Benthic macrofaunal biodiversity in relation to sediment sulfide concentration under salmon farms in southwestern New Brunswick, Bay of Fundy

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ABSTRACT

Chang, B.D., Cooper, J.A., Page, F.H., and Losier, R.J. 2017. Benthic macrofaunal biodiversity in relation to sediment sulfide concentration under salmon farms in southwestern New Brunswick, Bay of Fundy. Can. Tech. Rep. Fish. Aquat. Sci. 3202: v + 71 p.

Benthic sediment samples were collected under two marine salmon farms (sites A & B) and a reference site (C) in southwestern New Brunswick (SWNB), Bay of Fundy. Triplicate grab samples were taken at 6 stations at each site. Macrofaunal biodiversity and total free sulfide concentration were measured in each grab. Mean sulfide concentrations per station ranged from 139-1266 µM at farm A, 650-3550 µM at site B, and 67-129 µM at reference site C. Univariate diversity indices (number of taxa, Shannon diversity index, Margalef's species richness index, and Pielou's evenness index) indicated that the macrofaunal biodiversity under both farms was impacted by organic enrichment, with higher impacts at the larger farm (site B). There were clear indications of adverse effects on benthic macrofaunal biodiversity at sulfide concentrations above ~1500 µM and some indications of impacts at lower sulfide concentrations. A simple linear regression between the number of taxa and the sulfide concentration per station was not significant ($p=0.11$). Macrobenthic diversity was highest in sediments with intermediate sulfide concentrations, and lowest in sediments with higher sulfide concentrations. The biodiversity under reference site C was lower than under moderately enriched site A for some indices. *Capitella* spp. were low in abundance or absent at site C, while at the two farms, their abundance increased at intermediate sulfide concentrations, then decreased at higher sulfide concentrations. Sample variability associated with elevated sulfide concentrations indicated that sample size was an increasingly important considerations when evaluating relative impacts at higher concentrations. Ordination of the biodiversity data through multi-dimensional scaling (MDS) indicated that reference site C was distinct from the two farm sites, while there was some overlap between the two farm sites. *Capitella* spp. was the taxon that contributed the most to similarity within the farm sites (but not at the reference site) and to dissimilarity between all pairs of sites. There were also significant differences in biodiversity among sulfide classes. *Capitella* spp. was the taxon that contributed most to similarity within sulfide classes (except Oxic A) and to dissimilarity between pairs of sulfide classes (except Oxic A–Hypoxic B). The results generally agree with previous studies on the relationship between biodiversity and sediment sulfide concentration at fish farms in SWNB. Studies on the effects of sampling unit size and the level of taxonomic indentification on biodiversity measures are included as appendices.

RÉSUMÉ

Chang, B.D., Cooper, J.A., Page, F.H., and Losier, R.J. 2017. Benthic macrofaunal biodiversity in relation to sediment sulfide concentration under salmon farms in southwestern New Brunswick, Bay of Fundy. Can. Tech. Rep. Fish. Aquat. Sci. 3202: v + 71 p.

Des échantillons de sédiments benthiques ont été recueillis sous deux fermes salmonicoles marines (sites A et B) et dans un site de référence (site C) dans la baie de Fundy, au sud-ouest du Nouveau-Brunswick. Trois échantillons ponctuels ont été prélevés à six endroits différents de chaque site. La biodiversité de la macrofaune et la concentration totale de sulfure libre ont été

mesurées pour chaque échantillon. Les concentrations moyennes de sulfures par emplacement variaient de 139 à 1266 µM à la ferme A, de 650 à 3550 µM à la ferme B, et de 67 à 129 µM au site de référence C. Les indices de diversité à une variable (le nombre de taxons, l'indice de diversité de Shannon, l'indice de richesse spécifique de Margalef et l'indice d'équitabilité de Pielou) indiquent que l'enrichissement organique a eu des effets sur la biodiversité de la macrofaune sous les deux fermes et que l'incidence est plus élevée à la ferme la plus grande (site B). Des éléments indiquent clairement que des concentrations de sulfures supérieures à 1500 µM ont des effets nocifs sur la biodiversité de la macrofaune benthique et certains éléments indiquent qu'il y a des effets à de plus faibles concentrations. Une régression linéaire simple entre le nombre de taxons et la concentration de sulfure par station n'a pas été significative ($p=0.11$). La diversité macrobenthique la plus élevée se trouvait dans les sédiments aux concentrations de sulfures moyennes, et la diversité macrobenthique la plus faible, dans les sédiments aux concentrations élevées. Pour certains indices, la biodiversité dans le site de référence C était plus faible que dans le site A modérément enrichi. *Capitella* spp. étaient en faible abondance ou absents au site C. En revanche, aux deux fermes, leur abondance était supérieure à des concentrations de sulfures moyennes, mais était moins importante à des concentrations élevées. La variabilité des échantillons associée à des concentrations élevées de sulfure a indiqué que la taille de l'échantillon était un facteur de plus en plus important dans l'évaluation des effets relatifs à des concentrations élevées. L'ordination des données sur la biodiversité par le positionnement multidimensionnelle a indiqué que le site de référence C était distincte des deux fermes, alors qu'il y avait un certain chevauchement entre les deux fermes. *Capitella* spp. forment le taxon qui a le plus contribué aux similitudes entre les sites aquacoles (mais pas pour le site de référence) et aux différences entre toutes les paires de sites. Il y avait aussi des différences importantes dans la biodiversité selon les classes de sulfure. *Capitella* spp. sont également le taxon qui a le plus contribué aux similitudes selon des classes de sulfure (sauf oxique A) et aux différences entre des paires de classes de sulfure (sauf oxique A-hypoxique B). De manière générale, les résultats sont en accord avec les études précédentes sur le lien entre biodiversité et concentrations de sulfures dans les sédiments des exploitations aquacoles du sud-ouest du Nouveau-Brunswick. Inclus en annexe sont des études sur les effets de la taille de l'unité d'échantillonnage et le niveau de l'identification taxonomique sur les mesures de la biodiversité.

INTRODUCTION

Atlantic salmon (*Salmo salar*) have been farmed in the coastal waters of southwestern New Brunswick (SWNB), Bay of Fundy since 1978. There are currently over 90 licensed farms, of which approximately half are actively farming salmon at any given time. The Environmental Management Program (EMP) for the Marine Finfish Cage Aquaculture Industry in New Brunswick requires annual benthic monitoring of all approved fish farms between 1 August and 31 October (NBDELG 2012a,b). The goal of the EMP is to evaluate the condition of the sediments under marine finfish farms, using sediment sulfide concentration (total free S^{2-}) as the indicator of the state of the benthic community, as recommended by Wildish et al. (1999, 2004a). Each farm is rated based on the monitoring results, according to Table 1.

Previous studies have shown that organic enrichment due to salmon farms can have impacts on the benthic macrofaunal community in SWNB (Lim 1991; Pohle et al. 1994, 2001). The use of geochemical indicators, such as sediment sulfide, for monitoring programs has been promoted largely because of the lower cost and shorter analysis time, in comparison to benthic macrofaunal community analysis (Henderson & Ross 1995; Wildish et al. 2001a, 2001b, 2004a; Schaaning & Hansen 2005). The short time to obtain results from sulfide monitoring (usually within one day, compared to weeks or months for benthic community analysis) also means that remedial responses can be quickly implemented if elevated sulfide concentrations are found (NBDELG 2012a). Sediment sulfide concentration has become a key parameter in monitoring programs for salmon farming in several jurisdictions (Wilson et al. 2009). However, some authors report that geochemical measures (such as sulfides) are not always good indicators of impacts on benthic biodiversity (Henderson & Ross 1995; Carroll et al. 2003; Keeley et al. 2013).

The relationship between sediment sulfide concentration and the macrobenthic community under salmon farms has been reviewed in Hargrave et al. (2008) and Hargrave (2010). Data on this relationship were previously examined at several farms in SWNB (Hargrave et al. 1995, 1997; Wildish et al. 2001a, 2001b, 2002, 2004b, 2005), as well as from British Columbia (Brooks & Mahnken 2003) and New Zealand (Keeley et al. 2013). The present study conducted intensive sampling of sediment sulfide and benthic biodiversity under two active salmon farms and one reference location in SWNB. Diversity index values will change with increased sampling effort (Clarke & Warwick 2001). For the number of taxa, as the sampling effort increases (in a homogeneous habitat), so will the number of species found, eventually reaching an asymptote; however, it has been noted that in most marine sediment studies, the asymptotic values are not reached (Clarke & Warwick 2001; Ugland et al. 2003).

A preliminary summary report on this study was previously published (Chang et al. 2011a). The present report includes additional data and analyses to improve understanding of the relationship between sulfide and benthic diversity at locations exposed to a range of benthic impact measured by the current standards for elevated sediment sulfide (see Table 1, NBDELG 2012a). In addition the sensitivity of commonly reported biodiversity indices is examined with respect to site variability (as indicated by sediment sulfide concentration), level of impact, sample size, and taxonomic level of identification.

METHODS

Study sites

Two active Atlantic salmon (*Salmo salar*) farms (sites A and B) in the Letang area (45° 02' N, 66° 51' W) in SWNB were selected for this study. Site A was growing salmon only, while site B was a pilot for integrated multi-trophic aquaculture (IMTA), growing mussels (*Mytilus edulis*) and kelp (*Alaria esculenta* and *Saccharina latissima*) adjacent to salmon cages (Fig. 1). Site A began operating in 1989, but had been fallow for ~2.5 yr prior to stocking salmon smolts in August 2007. Site B began operating in 1995, and had been fallow for 4 months prior to stocking salmon smolts in October 2007. Site B was located ~5 km southwest of site A. Reference site C was located ~0.8 km east of site B and ~4 km southwest of site A; the nearest active salmon farm to site C was ~0.7 km to the northeast. Sites A and C were relatively flat and similar in depth, while site B was slightly deeper, with depth increasing from northeast to southwest (Fig. 1). Sites A and C were sampled on 27 October 2008 and site B was sampled on the following day. Site information is presented in Table 2.

The water temperature was not measured at the time of sampling. However, the temperature was 10.5°C at ~1 m above the seafloor at a location 0.5 km northwest of site A on 28 October 2008 (M.M. LeGresley, St. Andrews Biological Station, pers. comm.). Current velocity data were not obtained at the study sites at the time of sampling. However, data were obtained from 70-d current meter deployments in June-August 2009 near sites A & B (120-180 m from the nearest sampling stations) and a 35-d deployment in July-August 2010 near site C (320 m from the nearest sampling station). Mean current speeds at 3 depth levels (near surface, mid-water, and near bottom) were: 15.4, 15.1, and 16.2 cm s^{-1}, respectively, near site A; 7.8, 7.5, and 8.5 cm s^{-1} near site B; and 12.3, 9.6, and 8.9 cm s^{-1} near site C (R.J. Losier, unpublished data).

Field sampling

Benthic sediment samples were collected using a modified Hunter-Simpson grab (Hunter & Simpson 1976) which collected sediment samples 16 × 15 cm in surface area (0.024 m^2). The grab had a protective cover, to minimize disturbance to the sediment surface layer during retrieval. This small grab was used because it could be deployed from a smaller vessel which could manoeuvre and precisely sample between fish cages. The grab was able to sample sediment to a depth of 4-15 cm; the maximum sample size was approximately 1500 ml (~2 kg wet weight of soft sediment). Triplicate grab samples were taken within a few minutes of each other, at each of six stations at each site (Fig. 1). At farm sites A and B, the six sample stations were at the four corners of the cage array (i.e. at the outer edge of each of the four corner cages), plus two stations near the middle of the cage array. The 6 sample stations at reference site C were arranged in a similar spatial distribution (Fig. 1). From each grab sample, three spatially-scattered 5-ml syringe subsamples were collected from the top 2 cm of sediment for sulfide analysis. The subsamples were kept on ice for transport to the laboratory, and stored at ~5°C. All subsamples were analyzed for total free sulfides (S^{2-}) within 1 d of sampling, using the method described by Wildish et al. (1999, 2004a).

Sediment grain size data were not obtained; however all 3 sites had soft sediments. Data collected at site A in 1990 and 1994 indicated that the sediments were predominantly sand and mud (~43% silt-clay), with a peak grain size in the fine sand range (Milligan 1994; Hargrave et al. 1995). Data collected at site C in 1990 indicated that the sediments were predominantly mud (~97% silt-clay), with a peak grain size in the silt range (Milligan 1994). No quantitative sediment data were available at site B; unpublished observations during the annual regulatory monitoring of farms in 2008 described the sediments under this farm as fine grain. To account for the absence of grain size information, the wet weight of each grab sample was used to standardise univariate indices on a per kg of sample basis, as well as to ascertain gross changes in sediment softness within and among sites.

Sediment from the grab samples (minus the small subsamples removed for sulfide analyses) was collected in 2-L plastic containers and refrigerated for subsequent taxonomic composition analysis. The sediment samples were passed through a series of small mesh sieves in order to retain only material greater than 330 µm. This material was fixed in 10% buffered formalin for 72 h, then decanted and transferred to water for one week, and then decanted again and transferred to 50% isopropanol for final preservation. Identification of all individual animals in each sample was performed to the lowest taxonomic unit possible (in most cases to the species level). Taxonomic names and classifications were obtained from the World Register of Marine Species (WoRMS Editorial Board 2016). Species accumulation curves were estimated per site, based on the number of taxa accumulated for each subsequent sample randomized 10 times (Seaby & Henderson 2007). A logarithmic fit of this curve was used to calculate the number of new taxa (ΔT) observed if an additional sample was taken. Estimates of ΔT for 3 and 18 samples were used to assess sufficient sampling as a percentage of information gained.

Biodiversity data analysis

For the biodiversity analyses, the abundance data from the triplicate samples at each station were pooled. Univariate indices of diversity, species richness, and community similarity were calculated for each station using PRIMER v6 software (Clarke & Warwick 2001; Clarke & Gorley 2006). The following univariate biodiversity indices were calculated:

Standardised number of individuals: N (kg^{-1})

Number of taxa: T (This value is typically expressed as S, but we recognise that identification to species is not always possible, and the lowest taxon T is more representative of our data; we use S to represent concentration of free dissolved sulfide)

Shannon (or Shannon-Wiener) diversity index: $H' = -\sum_{i=1}^{T} p_i \ln(p_i)$

Margalef's species richness index: $d = (T-1)/\ln(N)$

Pielou's evenness index: $J' = H'/H'_{max} = H'/\ln(T)$

where p_i is the proportion of the total count due to the ith species. Indices such as number of taxa, Shannon, Margalef, and Peilou assume certain parameters of the data exist in order to reliably detect changes associated with habitat and environment change. Sufficient sampling effort (fully or near fully censused) is assumed per comparison unit for all such indices, and for many indices beyond the number of taxa, the data requires a log normal abundance distribution (Seaby & Henderson 2007). In many cases (especially in marine sediment) these parameters are not fully met (Clarke and Warwick 2001; Ugland et al. 2003). Intensive sampling (within and among sites) was used to investigate the effects of sample size and species abundance distribution on such indices relative to sulfide concentration.

In Appendix C we examined the effect of sampling unit size on the various diversity indices. To do this, we derived a range of sampling unit sizes through consolidation of data from individual grab samples. The sampling units were: individual grabs (18 grabs per site); stations (3 grabs combined per station; 6 stations per site); station pairs (6 grabs combined per station pair; 3 station pairs per site); and entire sites (18 grabs combined per site; one unit per site). Diversity indices were then calculated using the abundance data for these different sampling units and compared against sediment sulfide concentrations.

All univariate diversity indices and multivariate analyses were calculated using the lowest taxonomic level identified for each individual, which in most cases was the species level. Clarke & Warwick (2001) noted that, in many marine macrobenthos pollution studies, it has been found that little information is lost when species data are aggregated to higher levels. In Appendix D, we investigated the impact of aggregating the abundance data to higher taxonomic levels.

We also examined selected indicator species. An increased abundance of *Capitella* spp. is often used as an indicator of organic pollution in sediments (Pearson & Rosenberg 1978; Wildish & Pohle 2005). The bivalves *Ennucula delphinodonta* (formerly *Nucula delphinodonta*) and *Nucula proxima*, have been identified as indicators of low level organic enrichment in SWNB sediments (Pohle et al. 2001); in our study, we combined the abundances of these 2 species. Examing the presence of these taxa relative to sediment sulfide concentrations would improve our understanding of the benthic diversity response to aquaculture.

Statistical comparisons among the 3 sites and among sulfide classes were performed for each univariate index (using values calculated for the combined abundance data from triplicate grab samples at each station) by permutational analysis of variance computed using PERMANOVA+ for PRIMER (using resemblance matrices of Euclidean distances among samples for each diversity index); the probabilities for paired comparisons were not corrected for the possibility of rejecting null hypotheses by chance when performing multiple comparisons (Anderson et al. 2008).

Simple linear regressions between sulfide concentration (ln-transformed) and each of the univariate indices were calculated using the DISTLM routine in PERMANOVA+ (using the resemblance matrix of Euclidean distances among samples based on each index alone).

Values for univariate indices were compared to results from previous studies which have included data on biodiversity indices and sediment sulfide concentration at SWNB salmon farms

(Wildish et al. 2001a, 2001b, 2004b). Two other reports included species abundance data (as well as sulfide data) from SWNB (Wildish et al. 2002, 2005); we used the abundance data from these studies to calculate diversity indices.

We also compared results for the number of taxa (T) and the Shannon diversity index (H') with regression equations for empirical relationships between these indices and the concentration of free dissolved sulfide (S) in sediments from Hargrave (2010):

$$T = 104 - 11.4 \ln(S)$$
$$T = 89.8 - 9.5 \ln(S)$$

$$\ln(H') = 1.37 - 0.00022\, S$$
$$\ln(H') = 1.23 - 0.00017\, S$$

Ordination of the data through non-metric multi-dimensional scaling (MDS) was used as a means to examine similarities and dissimilarities in species composition among sites relative to sulfide concentration. This method has been recommended for studies of community responses to abiotic gradients (Clarke & Warwick 2001; Clarke & Gorley 2006). In the resulting 2-dimensional MDS plots, the inter-point distances are related to the rank order of dissimilarities between samples; stress levels <0.1 indicate good ordination, with little prospect of misleading interpretation (Clarke & Warwick 2001). MDS analyses were conducted using PRIMER v6 software, based on the Bray-Curtis resemblance matrix after square root transformation of the abundance data per station, run for 50 random restarts. Statistical comparisons among sites and among sulfide classes were performed on this resemblance matrix using PERMANOVA+. The relationship between sulfide concentration (ln-transformed) and this resemblance matrix was examined using the DISTLM routine in PERMANOVA+. The SIMPER routine (in PRIMER) was used to determine which taxa contributed the most to similarity among stations within sites and dissimilarity between pairs of sites, as well as to similarity among stations within sulfide classes and dissimilarity between pairs of sulfide classes (using square root transformed abundance data).

RESULTS

Grab sample sizes

The number of individuals per grab was not correlated with the sediment wet weight for the 18 grabs obtained within each site (Fig. 2). Similarily, the number of taxa per grab was not correlated with the sediment wet weight of grabs (Fig. 3). The mean wet weights of individual grab samples at each site (18 samples per site) were (mean ± SD): site A = 1.4 ± 0.31 kg (range: 0.6-1.9 kg); site B = 1.3 ± 0.28 kg (range: 0.6-1.9 kg); and site C = 1.4 ± 0.14 kg (range: 1.0-1.7 kg). There was no significant difference in the mean wet weight of grab samples among the 3 sites ($p=0.35$).

The accumulated number of taxa observed within each site (Fig. 4) estimated that incremental gains of taxa after 3 grabs (station) would reveal an 18-44% increase in new taxa, as compared to

gains after 18 grabs (site) would accumulate less than 2% of the total number of taxa that were observed (Table 3). The number of samples required to observe less than a 1% accumulation of new taxa was 23 for Site A, 29 for Site B, and 22 for Site C (Table 3). Further analyses on the effect of sample unit size on diversity measures are reported in Appendix C.

Sediment sulfide concentration

Mean sediment sulfide concentrations per station at the three sites are presented in Table 4 and Fig. 5a; individual measurement data are presented in Appendix A. There were significant differences in sulfide concentrations among the 3 sites and among stations within sites ($p=0.001$); differences between site pairs were probably significant for all 3 pairs (based on uncorrected p values; Table 5). At low mean sulfide concentrations, standard deviations were also low, but as the mean sulfide concentration increased, the standard deviation also increased (but with much variability, especially at higher sulfide means); however, there was no clear relationship between the mean sulfide concentration and the coefficient of variation (Fig. 5b).

At reference site C, the mean sediment sulfide concentrations were consistently low: the site mean was 92 µM (Oxic A), with station means ranging from 67-129 µM (Oxic A) and grab sample means ranging from 44-198 µM (Oxic A).

At site A, the mean sediment sulfide concentration was 793 µM (Oxic B), with station means ranging from 139-1393 µM (Oxic A to Oxic B) and grab sample means ranging from 127-1970 µM (Oxic A to Hypoxic A). All of the site A station means were higher than the highest station mean at site C. The sulfide concentration per station at site A was not correlated with the feeding rate (during the 4-week period leading up to the sediment sampling date), which varied widely among cages at this farm, nor with depth, which was relatively even under this farm: the highest mean sulfide concentration was at station A1, adjacent to the cage receiving the lowest amount of feed (see Fig. 1).

The mean sediment sulfide concentration at site B was 2257 µM (Hypoxic A), with station means ranging from 650-3550 µM (Oxic A to Hypoxic B) and grab sample means ranging from 363-6793 µM (Oxic A to Anoxic). All site B station means except B2 were classed as hypoxic (B2 was oxic); all station means except B2 were higher than the highest station mean at site A. The only values in the Anoxic range were from one grab at station B5 (at the centre of the site; Fig. 1); however, the mean sulfide concentration at this station was in the Hypoxic B class. The sulfide concentration per station at site B was not correlated with the feeding rate (during the 4-week period leading up to the sediment sampling date), which was relatively even among the cages at this farm (Table 2 and Fig. 1) or depth (which varied from 16-37 m under the cage array; see Fig. 1).

Univariate diversity indices

Univariate diversity index values per sampling station (based on the combined abundance data from the triplicate grab samples at each station) are presented in Table 4. Statistics for comparisons of diversity index values among sites and among sulfide classes are presented in Table 5. Graphs of the relationships between the indices and sulfide concentrations per station

are shown in Figs. 6-13. Significance values and the proportion of variation explained by simple linear regressions between the sulfide concentration (ln-transformed) and each diversity index (per station) are presented in Table 6.

Number of individuals

There was a significant difference in the number of individuals per station among the 3 sites ($p<0.01$); differences between site pairs were probably significant for A-B and A-C, and not significant for B-C (based on uncorrected p values). At reference site C, the number of individuals ranged from 67-170 kg^{-1} per station. At site A, the range was 162-290 kg^{-1} per station. At site B, the range was 20-277 kg^{-1} per station. There was also a significant difference in the number of individuals per station among sulfide classes ($p=0.03$). The numbers were highest where sulfide concentrations were above reference site levels, but <1500 µM (Fig. 6). The simple linear regression between the number of individuals and the sulfide concentration per station was not significant ($p=0.65$).

Number of taxa and most common taxa

A list of all identified taxa is included as Appendix B. There was a total of 123 taxa identified, of which 115 were identified to the species level (including 19 for which the species name was not determined). Of the 8 taxa not identified to the species level, 4 were in the class Polychaeta: genus *Capitella*; family Sabellidae; family Spionidae (excluding *Polydora* sp. and *Spiophanes bombyx*); and family Syllidae (excluding *Eusyllis* sp., *Exogone* sp., and *Syllis cornuta*). The other 4 exceptions were: family Tubificidae (excluding *Tubificoides benedii*; in the subclass Oligochaeta); order Harpacticoida; class Ostracoda; and subclass Acari. There were 107 different genera (for the 116 taxa identified to genus). Of the 123 taxa, 58 had <5 individuals in total (all 3 sites combined), including 29 with only 1 individual each. The results of a study into the effects of aggregating the taxa (to higher taxonomic levels) on diversity measures are presented in Appendix D.

The total number of taxa per site was 100 at site A, 70 at site B, and 69 at site C. The mean number of taxa per station was 53.3 at site A, 21.8 at site B, and 37.7 at site C. The most common taxon group at all 3 sites was Polychaeta, representing 56-84% of individuals per station at site A, 52-89% at site B, and 52-63% at site C (based on abundance per kg [wet weight] of sediment at each station). Oligochaeta was the next most common taxon group at sites A (3-22% of individuals per station) and C (16-28%), and the third most common taxon group at site B (0-16%). Bivalvia was the second most common taxon group at site B (4-26% of individuals per station), and the third most common taxon group at site A (7-16%) and C (8-16%). Together, these 3 taxon groups represented 86-98% of the individuals per station at the 3 sites.

There was a significant difference in the number of taxa per station among the 3 sites ($p<0.01$); the differences between site pairs were probably significant for A-B and A-C, but not significant for B-C. At reference site C, the number of taxa was within a relatively narrow range: 31-44 per station. The range at site A was higher, at 47-60 per station, while site B the number of taxa was mostly low, except at the one station classified as oxic (range: 7-58 per station). There was also a

significant difference in the number of taxa per station among sulfide classes ($p<0.01$). The highest values were at stations where the sulfide concentration was above reference site levels, but <1500 µM (Fig. 7). The simple linear regression between the number of taxa and the sulfide concentration per station was not significant ($p=0.11$).

The 3 most abundant taxa at each station are listed in Table 7. At site A, *Capitella* spp. was the most common taxon at the 3 stations classed as Oxic B (A1, A5 & A6) and at one station classed as Oxic A (A4); Tubificidae (excluding *T. benedii*) was the most common taxon at the other 2 stations classed as Oxic A (A2 & A3). At site B, *Capitella* spp. was the most common taxon at 4 of the 6 sampling stations (B1, B4, B5 & B6; all were Hypoxic A or B) and *Cossura longocirrata* (Polychaeta) was the most common at the other two stations (B2, classed as Oxic A; B3, classed as Hypoxic A). At reference site C (where all stations were classed as Oxic A), *C. longocirrata* was the most common taxon at station C5 and Tubificidae (excluding *T. benedii*) was the most common taxon at the other 5 stations.

The percent dominance of the 3 most abundant taxa (combined) per station fell within a fairly narrow range for all but 3 stations (Fig. 8). At all stations at sites A and C, plus 3 stations at site B (B1, B4 & B6), the 3 most abundant taxa represented 39-68% of the total abundance per station. At the other 3 stations (B2, B3 & B5) the percent dominance by the 3 most common taxa was much higher, 89-97%, largely due to *Capitella* spp. (81-86% of total abundance). These 3 stations were classed as Hypoxic A or B.

There was a significant difference in the abundance of *Capitella* spp. per station among the 3 sites ($p=0.02$); the differences between site pairs were probably significant for A-C and B-C, but not significant for A-B. *Capitella* spp. represented 25% of all individuals collected at site A, 31% of individuals at site B, and only 2% of individuals at site C. At reference site C the abundance of *Capitella* spp. was low, ranging from 0-7 kg^{-1} per station. At site A the range was much wider, including much higher numbers: 3-133 kg^{-1} per station. At site B the range was 2-67 kg^{-1} per station. There was also a significant difference in the abundance of *Capitella* spp. per station among sulfide classes ($p<0.01$). The abundance of *Capitella* spp. per station increased above background levels where the sulfide concentration reached ~500 µM (Fig. 9). The highest abundance among all stations was at A6 (133 kg^{-1}) at a sulfide concentration of 1266 µM. At sulfide concentrations >3000 µM the number of *Capitella* spp. was low (<30 kg^{-1}), but higher than at site C. The simple linear regression between the abundance of *Capitella* spp. and the sulfide concentration was significant ($p=0.02$).

There was no significant difference in the abundance of *Ennucula/Nucula* per station among the 3 sites ($p=0.93$). At reference site C the combined abundance of these species ranged from 4-9 kg^{-1} per station. At site A the range was 6-18 kg^{-1} per station. At site B the range was 0-52 kg^{-1} per station. The highest abundance of *Ennucula/Nucula* among all stations in the study was at B2 (52 kg^{-1}); this was the only oxic station at site B (Fig. 10). There was also no significant difference in the abundance of *Ennucula/Nucula* per station among sulfide classes ($p=0.38$). The simple linear regression between the abundance of *Ennucula/Nucula* and the sulfide concentration was not significant ($p=0.79$). *Ennucula/Nucula* peaked at lower sulfide concentrations than *Capitella* spp.; the highest abundances of *Ennucula/Nucula* were at stations where sulfide concentrations were in the Oxic A range, but higher than at the reference site.

Shannon diversity index (H')

There was a significant difference in *H'* per station among the 3 sites ($p<0.01$); differences between site pairs were probably significant for A-B and B-C, but not significant for A-C. At reference site C, *H'* was high and within a narrow range: 2.4-2.9 per station. The range of values at site A was similar: 2.3-3.1 per station. The range at site B was wider, including values much lower than at site C (range: 0.6-2.8 per station). There was also a significant difference in *H'* per station among sulfide classes ($p<0.01$). Where sulfide concentrations were <1000 µM, *H'* values were mostly within the range of values at site C; while where the sulfide concentration was greater, *H'* was lower than at site C. *H'* showed a general decreasing trend with increasing sediment sulfide concentration, although values showed high variability at higher sulfide concentrations (Fig. 11). The simple linear regression between *H'* and the sulfide concentration was significant ($p<0.01$).

Margalef's species richness index (d)

There was a significant difference in *d* per station among the 3 sites ($p<0.01$); differences between site pairs were probably significant for all 3 pairs. At reference site C, *d* was high and within a narrow range: 6.6-8.4 per station. The range at site A was slightly higher: 8.7-11.1 per station. Site B showed a wide range: 1.4-10.1 per station. There was also a significant difference in *d* per station among sulfide classes ($p<0.01$). The trend in *d* with sediment sulfide concentration (Fig. 12) was similar to that for *H'*, except that where sulfide concentrations were <1400 µM, *d* was higher than at site C. The simple linear regression between *d* and the sulfide concentration was not significant ($p=0.05$).

Pielou's evenness index (J')

There was a significant difference in *J'* per station among the 3 sites ($p<0.01$); differences between site pairs were probably significant for B-C, but not significant for A-B and A-C. At reference site C, *J'* was high and within a narrow range: 0.69-0.76 per station. The range at site A was similar: 0.58-0.77 per station. The range at site B was wider: 0.28-0.69 per station. There was also a significant difference in *J'* per station among sulfide classes ($p=0.01$). *J'* showed a decreasing trend with increasing sediment sulfide concentration up to ~2000 µM, but above that concentration *J'* showed wide variability (Fig. 13). The simple linear regression between *J'* and the sulfide concentration was significant ($p<0.01$).

Multi-Dimensional Scaling (MDS) analysis

The 2-dimensional MDS plot on square root transformed data (using the abundance data from the combined triplicate grab samples at each station) had a stress level of 0.05, indicating good ordination. All of the site C stations were in a small cluster (at the bottom left in Fig. 14) which did not overlap with stations at the two fish farms. Site A stations were in a separate, slightly larger, cluster at the top left. Site B stations were spread out both horizontally and vertically over the plot, with one station within the cluster of site A stations, but no overlap with site C stations. For the community structure (defined by the resemblance matrix of Bray-Curtis similarities of square root transformed abundance data) there was a significant difference among the 3 sites

($p<0.01$); differences between site pairs were probably significant for all 3 pairs (Table 5). There was also a significant difference in community structure (defined as above) among sulfide classes ($p<0.01$). The relationship between community structure and sulfide concentration was significant ($p<0.01$); the MDS bubble plot also indicated a relationship with sediment sulfide concentration (Fig. 14).

The taxa most contributing to similarity among stations within sites and to dissimilarity between pairs of sites are shown in Table 8. At site A, the taxon most contributing to similarity among stations was *Capitella* spp., followed by Tubificidae (excluding *T. benedii*). At site B, the taxon most contributing to similarity among stations was also *Capitella* spp., followed by *Mytilus edulis*. At site C, the taxon most contributing to similarity among stations was Tubificidae (excluding *T. benedii*), followed by *Cossura longocirrata*. Between sites A & B the taxa most contributing to dissimilarity among stations were Tubificidae (excluding *T. benedii*) and *Capitella* spp. (equally important). Between sites A & C the taxon most contributing to dissimilarity was *Capitella* spp., followed by *Tharyx* sp. Between sites B & C the taxon most contributing to dissimilarity was *Capitella* spp., followed by *C. longocirrata*.

The taxa most contributing to similarity among stations within sulfide classes and to dissimilarity between pairs of sulfide classes are shown in Table 9. *Capitella* spp. was the taxon most contributing to similarity among stations within all sulfide classes, except for Oxic A, where Tubificidae (excluding *T. benedii*) was the most important. The contribution of *Capitella* spp. to similarity was especially important for Hypoxic A (45%) and Hypoxic B (50%). *Capitella* spp. was also the taxon most contributing to dissimilarity among stations between sulfide class pairs, except for Oxic A–Hypoxic B, where *C. longocirrata* was the most important taxon.

DISCUSSION

Effect of sampling unit size on diversity measures

The distribution of grab wet weights per site appeared to be similar, with most samples capturing between 1 and 2 kg of sediment per grab (Fig. 2). The average total wet weight of sediment per grab was statistically the same among sites ($p=0.35$). In the absence of sediment characteristics such as grain size and total carbon, we inferred from this consistency in grab weight that the sediment type was at least similarly soft in all three locations. In addition, the number of individuals per grab (Fig. 2) or the number of taxa observed (Fig. 3) per grab were not affected by variation in sediment grab weights at these sites.

The relationship between the accumulated number of taxa and the number of grab samples within each site (Fig. 4) estimated an incremental increase of 18-44% new taxa after 3 samples (Table 3). In contrast, for all samples within a site ($n=18$), additional sampling effort would only result in an incremental increase of <2% (Table 3).

The number of samples required to achieve an incremental increase of less than 1% varied between sites with the reference site C requiring 22 samples, sites A 23 samples and site B 29 samples. This suggested a greater sample variability within the two aquaculture sites A and B.

As a result, pooling information for all 18 samples within a site would seem to be appropriate to achieve sufficient sampling required for parametric biodiversity indices such as Shannon, Margalef, and Peilou (Seaby & Henderson 2007). However, this site pooling of the data severely limits resolution to examine the effects of sulfide concentration on diversity, especially within a site. Using the combined abundance data from the triplicate samples at each station is a compromise in order to capture within site habitat variability measured by sulfide concentation. This issue is explored further in Appendix C.

Effect of taxonomic level on diversity measures

As has been reported in many macrobenthic studies (see Clarke & Warwick 2001), we found that aggregating taxa to the family level did not reduce the power to find significant differences among sites or among sulfide classes for the various diversity measures (see Appendix D). Similarly, aggregation to the family level did not reduce the significance of the relationships between sulfide concentration and the various diversity measures (see Appendix D). This suggests that taxonomic analysis to the species or genus levels is not required; identification only to the family level should result in savings in costs and time.

Sediment sulfide concentration and sources of variability

Site A had lower fish biomass and feeding rates and higher mean current speed than site B. Such conditions should favor lower sediment sulfide concentrations at site A compared to site B (Chang et al. 2013), and this was observed in our study. According to the classification system used in SWNB (NBDELG 2012a), the mean sediment sulfide concentrations for site A would have been classed as Oxic B and site B would have been classed as Hypoxic A (Table 1). Both farm sites had significantly higher sulfide concentrations than the reference site C, where they were 68-129 μM (Oxic A). The sulfide concentrations at site C were within background levels reported for SWNB, which are generally <300 μM (based on data collected at reference sites away from fish farms and other pollution sources reported in Hargrave et al. 1995 & 1997, and unpublished data collected since 2000 at new finfish farm sites prior to the start of operations).

Increasing variability was coincident with increasing sulfide concentration. Other studies in SWNB have shown that wide variations in sulfide concentration can often occur among replicate samples (Hargrave et al. 1995; Chang et al. 2011b). Therefore, the accuracy of the sulfide data, as a representation of the organic matter accumulation reflected in the benthic community data, depends on the number and location of subsamples taken for sulfide analyses (in our study, 3 spatially-scattered subsamples per grab sample). Another possible source of error is variation in sulfide measurement techniques: Brooks & Mahnken (2003) reported that subtle differences in protocols and/or techniques can results in significant differences in sulfide results. However, this should not have been a factor in our study, since all sediment sampling and sulfide measurements were conducted with the same people, equipment, and methods. A major factor in the increasing variability with increasing sulfide concentration is that the relationship between the electrode potential (measured by the sulfide meter/probe) and the sulfide concentration is semi-logarithmic. At low sulfide concentrations, a small variation in electrode potential translates into a small variation in sulfide concentration, but as the sulfide concentration increases, small

variations in electrode potential will translate into increasingly larger variations in sulfide concentration (Thermo Scientific Inc. 2007; Chang et al. 2014).

The similarity in grab performance coupled with differences in sediment sulfide concentrations observed at the 3 study sites indicated a change in habitat quality on similar bottom types. This was determined to be an appropriate circumstance to investigate relationships between sediment sulfide and diversity measures.

Relationships between sediment sulfide concentration and univariate diversity measures

Hargrave et al. (2008) proposed a classification system for the impacts of organic enrichment on marine sediments. In their nomogram, they associated Oxic A conditions with high biodiversity of benthic macrofauna, Oxic B with moderate biodiversity, Hypoxic with reduced biodiversity, and Anoxic with very low biodiversity. In our study, the benthic macrofaunal community showed impacts associated with organic enrichment (as measured by sediment sulfide concentrations), with higher impacts at site B (the larger farm), which also had higher sediment sulfide concentrations at most stations. However, at both farms (but especially site B), there was considerable variability in benthic impacts and sulfide concentrations among sampling stations, and the predicted decline in biodiversity from "high" to "moderate" in the transition from Oxic A and Oxic B conditions required further investigation.

In our study, there was a significant decline in biodiversity indices such as Shannon (*H'*) and Pielou (*J'*) related to increases in sulfide concentration (Table 6). However, linear regression explained less than half of the variation in the diversity indices. Simpler indices of biodiversity such as the number individuals or number of taxa showed no significant linear relationships with sulfide concentration (Table 6). Sulfide concentration explained approximately 35% of the variation in community structure, with a significant regression for the abundance of *Capitella* spp., but not for *Ennucula/Nucula* (Table 6). These indices revealed some increase at intermediate sulfide concentrations (above reference site levels but less than 1500 µM) before a decline at higher sulfide concentrations. Higher numbers of individuals and organic enrichment are often due to the abundance of indicator species, such as *Capitella* spp., which can show very high abundance at intermediate levels of organic matter input (see Wildish & Pohle 2005; Hargrave et al. 2008). Enhanced diversity at intermediate levels of disturbance is a well recognized ecological principle (Pearson and Rosenberg 1978, Wilkinson 1999). Sulfide concentrations between 750 and 1500 µm (Oxic B) appear to represent such an intermediate level of disturbance, when comparing our simplest indices of biodiversity from the low reference concentrations at site C with those observed at site A.

Despite variability with higher sulfide concentration and small sample sizes, there were significant differences among the sites. The number of individuals and number of taxa per station were higher in site A compared to either site B or C (Table 4). This is consistent with intermediate enrichment, but it is uninformative that the sites with the highest and lowest recorded sulfides (sites B and C) were not significantly different. The effect of biodiversity enhancement in site A and the increased variability in site C contributed to this result. Simple indices such as number of individuals and the number of taxa produced results contrary to our expectations for increased levels of sulfide.

The Shannon (H'), Margalef (d), and Pielou (J') biodiversity indices showed reduced values above sulfide concentrations of 1500 µm (Hypoxic A) and all three indicated at least one significant pair-wise difference among the three sites (Table 5). However, these indices did not agree in determining which pairwise sites were different. Shannon (H') indicated no pairwise difference between sites A and C (Table 5) and did not detect an effect of intermediate organic enhancement between sites C and A (Fig. 11). Margalef's index (d) indicated a significant difference in all pairwise site comparisons (Table 5). It not only detected the intermediate organic enhancement with an increased index for site A, but also consistently reported a reduced index associated with higher sulfide concentration despite small sample sizes (Fig. 12). Pielou's index (J') is different from other indices in being an evenness index. The increased amount of within site variability for site B is indicated by a pairwise difference between sites B and C, yet variability between sites A and B was not captured (Table 5). The parameters for this index require that samples come from the same habitat (Seaby & Henderson 2007), which was not necessarily met in the more impacted site B. The sampling must have a close estimate of T as it is required for the calculation of J'. Applying this type of index would be a poor choice to interpret site impact without a sufficient sample size for T. Further investigation as to the effects of sample size on these indices are explored in Appendix C to illustrate the loss of resolution with pooled sampling as a trade off for increased index consistency within a site.

Role of multi-dimensional scaling to discern changes in macrobenthic diversity and sediment sulfide concentration.

As illustrated, the application of univariate biodiversity indices to investigate impacts associated with increase sediment sulfide concentrations can produce contrary and even misleading results. This is especially true when sulfide measurements are more variable at higher concentrations and biodiversity is under-sampled. Confounding factors such as these are somewhat unavoidable with current measurement techniques and realistic sampling protocols. However multi-dimensional scaling (MDS) based on similarities in species composition (Clark and Warwick 2001) offered consensus and improved understanding of these relationships within the study sites.

At site A, the values for most indices indicated that macrobenthic diversity per station was mostly higher than, or similar to, reference site C. The abundance of *Capitella* spp. (Fig. 9), a well-known indicator of organic enrichment (Pearson & Rosenberg 1978; Wildish & Pohle 2005), indicated that there was some impact on the sediment macrobiota at site A, except at station A3 where the sulfide concentration was similar to the reference site. The MDS plot (Fig. 14) showed most of the site A stations clustered together, but separate from the reference site stations; the greatest separation from the reference site stations was in the 3 site A stations with the highest sulfide concentrations.

At site B (the larger farm, with lower mean current speed), most of the biodiversity indices indicated that the benthic community was more heavily impacted, with lower biodiversity in comparison to both reference site C and farm site A, except at station B2, where the sulfide concentration was in the Oxic A range.

Capitella spp. was the most important taxon for defining similarities and differences among the 3 sites (Table 8). This taxon made the highest contribution to similarity within each of the 2 farm sites (while at the reference site, *Cossura longocirrata* was the most important taxon), and to dissimilarity between pairs of sites. *Capitella* spp. was also the most important taxon for defining similarities and differences among sulfide classes. This taxon made the highest contribution to similarity within each sulfide class (except Oxic A, where Tubificidae [excluding *T. benedii*] was the most important taxon) and to dissimilarity between pairs of sulfide classes (except Oxic A–Hypoxic B, where *C. longocirrata* was the most important taxon).

The MDS results provided a much more consistent picture on the relationship between sulfide concentration and biodiversity despite variation within a site. Notable exceptions were stations B3, B4, and B6 (Fig. 14), which were all classified as Hypoxic A (Table 4). These 3 stations should have resolved closer to each other based on sulfide alone. The sample sulfide variability measured at station B3 (Fig. 5) indicated a large degree of error associated with this station, that would contribute to this type of inconsistency. Similarly, the relatively wide separation between the two Hypoxic B stations, B1 and B5 (Fig, 14), may be related to the large variability in sulfide concentration at B5 (Fig. 5).

Comparisons with data from other studies in SWNB

Reports by Wildish et al. (2001a, 2001b, 2004b) include sulfide concentrations and various macrobenthic biodiversity measures at or near other salmon farms in SWNB. Two other reports (Wildish et al. 2002, 2005) include species abundance data, which can be used to calculate biodiversity measures. Comparisons between values of the various diversity measures in our studies with other studies must be interpreted with caution, because of differences in sampling methodologies between studies. For example, our study used pooled triplicate Hunter-Simpson grab samples, with a surface area of 0.072 m^2 (0.024 m^2 per grab), which is larger in surface area than the wedge corer (0.026 m^2) used by Wildish et al (2001a, 2004b), but similar to the pooled triplicate wedge core samples (0.079 m^2) used by Wildish et al. (2002) and the 0.1 m^2 Hunter-Simpson grab used by Wildish et al. (2001b, 2005). The sieve mesh size also varies between studies: we used a 330 µm mesh, while 1000 µm has been used in most other studies in SWNB. Another factor affecting comparisons among studies, of course, will be differences due to natural variability in biota among different locations.

Summary information on other studies that have collected data on sediment sulfide concentration and macrobenthic biodiversity in SWNB is presented in Table 10. Wildish et al. (2001a) was conducted at an active farm (the mean sulfide concentration was Anoxic) and a reference site (Hypoxic A); diversity measures indicated high impacts at the farm and moderate impacts at the reference site. Wildish et al. (2001b) was at a farm that had been fallow ~9 months (Oxic B); diversity measures indicated no or low impacts. Wildish et al. (2002) collected monthly samples over a year near an active farm (~70-80 m away from the nearest cage; Oxic A to Oxic B), and at a farm that had been inactive ~2.5 years (Oxic A to Hypoxic A); diversity measures indicated no to moderate impacts at both sites. Wildish et al. (2004b) was conducted on two dates (about a year apart) at an active farm (Anoxic and Hypoxic A) and a reference site (Hypoxic A and Oxic A); diversity measures indicated high impacts at the farm and no impacts at the reference

site. Wildish et al. (2005) was at a new farm, not yet fully stocked at the time of sampling (Oxic A); diversity measures indicated no or low impacts.

Despite these caveats, data on univariate diversity indices and sulfide concentration in other studies in SWNB were consistent with our findings. The number of individuals (*N*) from other SWNB studies show no clear relationship with sulfide concentration (Fig. 15). Although our data showed some possible enrichment effects at at intermediate sulfide concentrations, data on the number of individuals observed in previous studies were not as clear. The generally higher abundance in our study (compared to previous studies) may be related to the smaller mesh size we used for sorting samples, as well as differences in sample sizes.

The number of taxa and sulfide concentration generally fit the empirical relationships derived by Hargrave (2010), but values from our reference site were all lower than predicted (Fig. 15), suggesting that the general relationship is more consistent with enrichment at intermediate sulfide concentrations (Pearson and Rosenberg, 1978).

Observations of the Shannon diversity index (*H'*) in our study and other studies with low to moderate sulfide concentrations were also less than predicted by the Hargrave (2010) equations (which were derived using data from British Columbia), and more consistent with a curve representing enrichment at intermediate sulfide concentrations (Fig. 15). In a study at New Zealand salmon farms, Keeley et al. (2013) also found that values of *H'* were less than predicted by the Hargrave (2010) equations.

The relationship for Margalef's species richness index (*d*) was very similar to that for *H'*. Pielou's evenness index (*J'*) based on available data (Wildish et al. 2001a, 2001b, 20002, 2005) did not show decreasing eveness among stations (except at very high sulfide concentration), but did illustrate an increase in the variability for this index with increasing sulfide concentration (Fig. 15). A result from insufficient estimates of *T*, it is a good example of increased sample variability encountered in sites that have higher sulfide concentrations.

Data for various diversity measures indicate that our two active farm sites were less impacted than the active farms studied by Wildish et al. (2001a & 2004b). However, our farm sites (especially site B) had higher impacts than farms in Wildish et al. (2001b, 2002 & 2005); these latter studies were at fallowed farms, new farms, or at locations not immediately adjacent to fish cages.

Capitella spp. are considered to be opportunistic species, which increase in abundance as organic enrichment increases, but disappear at higher levels of organic enrichment (Pearson & Rosenberg 1978; Wildish & Pohle 2005). In our study, the abundance of *Capitella* spp. was low at sulfide concentrations up to ~500 µM (Oxic A) and peaked at sulfide concentrations of 1000-1700 µM (Oxic B to Hypoxic A). The highest abundance per station was 133 kg^{-1} at station A6, where the mean sulfide concentration was 1266 µM (Oxic B). *Capitella* spp. abundance was mostly low where the sulfide concentration was >2500 µM. These results agree with laboratory studies by Cuomo (1985) which found that settlement and subsequent metamorphosis and survival of *Capitella* sp. occurred at sulfide concentrations of 100-1000 µM, while 10 000 µM was lethal (the study did not conduct tests between 1000 and 10 000 µM). Another laboratory study found no acute toxic effects on *Capitella* sp. larvae at sulfide concentrations up to

2000 µM (Dubilier 1988). In contrast to our results, studies at SWNB farms by Wildish et al. (2001a, 2002, 2004b, 2005) found that *Capitella capitata* was absent or very low in abundance at sediment sulfide concentrations up to 2500 µM, but was abundant at higher sulfide concentrations (>12 000 µM S^{2-}).

Wildish et al. (2001a) reported that *C. capitata* averaged >90% of the individual animals in samples collected under an active salmon farm, while in our study, *Capitella* spp. were less dominant, comprising 25% and 31% of individuals at the two active farms (compared to 2% at the reference site), although they were >80% of individuals at 3 site B stations. *C. capitata* was also the most abundant taxon at the farm site in Wildish et al. (2004b), but was found in only low numbers in Wildish et al. (2001b, 2002 & 2005); as noted above, these latter studies were at fallowed farms, new farms, or at locations not directly adjacent to fish cages. Various studies at salmon farms in SWNB have found abundances of *Capitella* spp. ranging from 0–30 000 m^{-2} (Pocklington et al. 1994; Hargrave et al. 1993, 1995, 1997; Lim 1991; Pohle et al. 1994; Wildish et al. 2001b, 2002, 2005), while in our study, the highest abundance per station was equivalent to ~7000 m^{-2}.

The abundance of *Ennucula delphinodonta* and *Nucula proxima* in our study confirms their preference for slightly enriched sediments, as was reported by Pohle et al. (2001). These bivalve species were low in abundance at the reference station (C) and at hypoxic stations at the farm sites. Highest abundances were at farm stations where sulfide concentrations were Oxic A, but above background levels; in contrast, other SWNB studies (Wildish et al. 2002, 2005) did not find peaks in *Ennucula/Nucula* abundance at these sulfide concentrations. Another species which has been suggested as an indicator of organic enrichment at fish farms is the bivalve *Nuculana temisulata*. In a 1994 study in SWNB, this species was found to be abundant at salmon farms, but absent at most reference sites (Hargrave et al. 1995); however, in our study, the only species found in this genus (*Nuculana* sp.) was rare or absent at all 3 sites.

The variability in the relationships between sulfide concentration and biodiversity measures can be due to several factors, including error in sulfide measurements and natural spatial variability in the macrobenthic community. Differences in current speed among sites may also affect the relationship (Keeley et al. 2013); lower current speeds, such at our site B, can be conducive to higher impacts. Another factor at farms which have caused significant impacts, but have subsequently fallowed or reduced production to mitigate the impacts, is that sediment geochemistry may have returned to normal oxic conditions, but benthic biology has not yet recovered (Henderson & Ross 1995; Macleod et al. 2004).

Implications for environmental management of salmon farms in SWNB

The environmental management program for finfish farming in SWNB is based on maintaining benthic biodiversity (or fish habitat) under farms, using sediment sulfide concentration as the indicator (NBDELG 2012a). The management framework states that fish habitat concerns increase when sediments become hypoxic (i.e. ≥1500 µM S^{2-}) and that the most severe losses in macrofaunal biodiversity occur at ≥3000 µM S^{2-}. Hence, no site management responses are required if sediment sulfide concentrations remain <1500 µM (using the mean sulfide concentration of all samples taken during the annual monitoring program). When sulfide

concentrations reach 1500 µM, a site management response is required, but it is relatively minor: adjustments to operational best management practices must be made, including examination of data on feed rates and fish growth per cage, and reviewing staff training, equipment maintenance, and site cleaning practices. However, when sulfide concentrations reach 3000 µM, the required site management response is more significant: additional (more intensive) monitoring, plus additional best management practices which may include early harvesting of some cages. As sulfide concentrations increase further, more severe site management responses are required.

The data from our study confirm that clear adverse impacts were occurring in the macrobenthos at sulfide concentrations ≥3000 µM, but also indicate that impacts began at ~1500 µM (the upper limit of Oxic B), with some occurring at even lower sulfide concentrations.

The environmental management framework in SWNB (NBDELG 2012a) uses thresholds based on the average sediment sulfide concentration from 2-8 designated sampling stations per farm (depending on the size of the farm). Samples are collected under the edges of cages holding higher biomasses of fish, during the late summer or fall, when impacts would be expected to be highest (NBDELG 2012b). These sampling stations differ somewhat in number and location from those in our study; however, if we were to classify our sites using the mean sediment sulfide concentration from all measurements at each site, then site A would be classed as Oxic B, site B as Hypoxic A, and reference site C as Oxic A. This would mean that no site management response would be required for site A, while site B would only require minor adjustments to the operational best management practices. Some impacts on biodiversity were occurring at stations within site A, but under the current sulfide threshold, no site management responses would be required at this farm. There were clear adverse impacts on the biodiversity at most stations within site B, yet the only site management response that would be required at this farm would be to adjust site management practices, with no specific requirement to reduce feeding or biomass. At the same time, caution must be used when setting thresholds, due to the high variability in the data (especially at higher sulfide concentrations) and in the relationships between the sulfide concentration and the biodiversity measures. Additional site management responses when sulfide concentrations reach a certain level may be difficult to justify; further sampling may be warranted to confirm the elevated concentrations.

CONCLUSIONS

This study included data from two active salmon farms (plus a reference site), where sulfide concentrations (station means) ranged from oxic to hypoxic. The biodiversity at stations at farm site A indicated no to moderate impacts, while at site B there were no to high impacts, and at reference site C there were no apparent impacts at all stations. Our data confirm that sediment sulfide concentration can serve as an indicator of impacts on the benthic macrofaunal community, but there can be considerable variability in the relationship, especially at intermediate and higher levels of organic enrichment. Adverse impacts such as fewer individuals, fewer taxa, and reduced diversity were clearly occurring in hypoxic sediments: most diversity indices indicated that the transition from background to elevated impacts was at sulfide concentrations around 1000-1500 µM. However, the abundance of *Capitella* spp. and

Ennucula/Nucula suggested that the transition to elevated impacts may start at slightly lower sulfide concentrations, around 500-1000 µM. At hypoxic sulfide concentrations (>1500 µm), there was generally lower biodiversity, but also an increase in index variability that is associated with increased habitat variability and insufficient sampling at the station (3 grab) level.

Different biodiversity measures showed different responses to changing sulfide concentrations. As noted by Keeley et al. (2012), many individual indicators of biodiversity may not be sensitive to changes under all conditions; hence, use of several biodiversity measures is recommended. Some diversity indices varied with increasing sample size (derived by combining data from individual grab samples): as the sampling unit size increased, the number of taxa and Margalef's species richness index (*d*) showed large (non-linear) increases; the Shannon diversity index (*H'*) also increased (non-linearly), but to a lesser degree; however, there was relatively little change in the number of individuals, the number of *Capitella* spp., and Pielou's evenness index (*J'*). Individual grab samples were not sufficiently large to adequately represent the benthic macrofaunal community. Pooling of the samples did improve index reliability, but at the expense of fine scale understanding of the relationships between sulfide concentration and diversity.

The relationship between sediment sulfide concentration and benthic macrofaunal biodiversity can be affected by variability in sulfide measurements as well as undersampling in variable habitats (Seaby & Henderson 2007). Despite the variability in the relationship between sediment sulfide concentration and benthic macrofaunal diversity, sulfide can be a useful monitoring tool, especially given its cost-effectiveness and the quick turnaround for results. The high variability in the relationship means that consideration of inherent error in high sulfide measurements and reduced certainty of sampling taxa under increased habitat heterogeneity must be taken into account when setting threshold sulfide concentrations for regulatory responses. A tiered monitoring approach, with increased sampling intensity at higher sulfide concentrations, may be warranted.

Collection of larger samples would provide more confidence that the biodiversity is adequately represented. This could be accomplished using larger grab samplers; however, the size of grab used would be limited by the need to be able to deploy from a small boat which can manoeuvre and sample between cages at a fish farm. An alternative would be taking additional replicates at each sampling station; the replicates could then be combined into larger sampling units.

The results confirm previous findings (see Clarke & Warwick 2001) that taxonomic identification to species or genus is probably not required in macrobenthic pollution studies. Identification to family can result in savings in costs and time, without significant loss of power to distinguish between sites or sulfide classes for most diversity measures.

Additional data should be collected at other farms (covering a range of organic enrichment, current speeds, sediment types, and farm sizes) to confirm the general applicability of the findings from this study.

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Table 1. Site classifications based on sediment sulfide concentrations in the Environmental Management Program (EMP) for finfish farms in southwestern New Brunswick (NBDELG 2012a).

Site classification	Sediment sulfide concentration (μM)	Effects on marine sediments
Oxic A	<750	Low effects
Oxic B	750–1499	Low effects
Hypoxic A	1500–2999	May be causing adverse effects
Hypoxic B	3000–4499	Likely causing adverse effects
Hypoxic C	4500–5999	Causing adverse effects
Anoxic	≥6000	Causing severe damage

Table 2. Background information for 3 study sites: 2 salmon farms (A & B) and a reference site (C). The number of fish and fish biomass are estimates for 25 October 2008. Feeding rates are averages of weekly rates during the period 28 September to 25 October 2008. Depths are below lowest normal tide; the mean tidal range in the study area is ~6 m and the large tidal range is ~8 m (data from the Canadian Hydrographic Service, Dartmouth, NS).

Site	A	B	C
Stocking date	Aug 2007	Oct 2007	Reference site
Number of cages	10	20	–
Cage circumference (m)	70	100	–
Total number of salmon	144 550	489 780	–
Total salmon biomass (t)	294	620	–
Biomass per cage (kg): mean	29 450	31 010	–
Biomass per cage (kg): range	18 810–36 760	19 130–37 710	–
Total feeding rate (t per week)	18 650	45 000	–
Feed per cage (kg per week): mean	1 870	2 250	–
Feed per cage (kg per week): range	990–2 270	1 750–2 540	–
Depth range at sampling stations (m)	15–19	19–34	16–18

Table 3. Estimated logistic equation for accumulation curves based on 18 grab samples per site, adjusted R^2 for the equation, the percent of incremental gain in the number of accumulated taxa for the next sample (%ΔT), and the number of samples required to achieve <1% increase in the accumulated taxa ($\Delta T < 1\%$). T = number of taxa; x = number of samples.

Site	Estimated equation	Adjusted R^2	Percent incremental gain after 3 samples (% ΔT)	Percent incremental gain after 18 samples (% ΔT)	Number of samples for $\Delta T < 1\%$
A	$T_A = 21.90 \ln(x) + 32.78$	0.99	18	1.2	23
B	$T_B = 21.20 \ln(x) + 4.89$	0.96	44	1.7	29
C	$T_C = 15.08 \ln(x) + 24.31$	0.99	18	1.2	22

Table 4. Univariate diversity indices for benthic macrofuana at two salmon farms (sites A & B) and a reference site (C). The indices are: the number of individuals per kg (wet weight) of sediment (*N*); the number of taxa (*T*); the Shannon diversity index (*H'*); Margalef's species richness index (*d*); and Pielou's evenness index (*J'*). Six stations were sampled at each site. Sulfide concentrations are means of 9 measurements per station. Diversity indices were calculated using the combined abundance data from triplicate grab samples at each station.

Station	Mean sulfide concentration (μM)	Sulfide class	Univariate diversity indices				
			N (kg^{-1})	T	H'	d	J'
Site A							
A1	1 393	Oxic B	162	47	2.4	9.0	0.61
A2	505	Oxic A	290	57	3.0	9.9	0.75
A3	139	Oxic A	166	53	3.1	10.2	0.77
A4	516	Oxic A	269	53	2.8	9.3	0.70
A5	938	Oxic B	200	60	2.8	11.1	0.69
A6	1 266	Oxic B	278	50	2.3	8.7	0.58
Means	**793**	**Oxic B**	**228**	**53**	**2.7**	**9.7**	**0.68**
Site B							
B1	3 256	Hypoxic B	20	9	0.9	2.7	0.39
B2	650	Oxic A	277	58	2.8	10.1	0.69
B3	2 037	Hypoxic A	75	23	2.1	5.1	0.68
B4	1 500	Hypoxic A	78	7	0.6	1.4	0.28
B5	3 550	Hypoxic B	77	22	2.0	4.8	0.66
B6	2 550	Hypoxic A	80	12	0.7	2.5	0.29
Means	**2 257**	**Hypoxic A**	**101**	**22**	**1.5**	**4.4**	**0.50**
Site C							
C1	68	Oxic A	129	41	2.8	8.2	0.76
C2	67	Oxic A	170	44	2.9	8.4	0.76
C3	123	Oxic A	67	33	2.6	7.6	0.74
C4	83	Oxic A	97	31	2.4	6.6	0.69
C5	129	Oxic A	108	34	2.7	7.0	0.76
C6	82	Oxic A	148	43	2.8	8.4	0.75
Means	**92**	**Oxic A**	**120**	**38**	**2.7**	**7.7**	**0.74**

Table 5. Probabilities for significance tests comparing sulfide (*S*) classes and study sites (farms A & B and reference site C) for sulfide concentration and various diversity measures. For each sampling station, the abundance data from triplicate grab samples were combined. For sulfide concentration, univariate diversity indices, and selected taxa, permutational analyses of variance were computed using PERMANOVA+ for PRIMER (using resemblance matrices of Euclidean distances among samples for each measure). For community structure, PERMANOVA+ was run on the resemblance matrix of Bray-Curtis similarities for square root transformed abundance data. Probabilities in bold italics in main tests are significant ($p<0.05$). The probabilities for paired comparisons are exact permutational values, not corrected for the possibility of rejecting null hypotheses by chance when performing multiple comparisons; for this reason, significance is not shown for the paired tests.

	Main tests		Pair-wise tests between sites		
Diversity measure	*S* class	Sites (A-B-C)	A-B	A-C	B-C
Sulfide (ln-transformed)	–	***<0.01***	0.02	<0.01	<0.01
Univariate diversity indices					
No. of individuals (*N*)	***0.02***	***<0.01***	0.02	<0.01	0.69
No. of taxa (*T*)	***<0.01***	***<0.01***	0.01	<0.01	0.08
Shannon (*H'*)	***<0.01***	***<0.01***	0.02	0.87	0.01
Margalef (*d*)	***<0.01***	***<0.01***	0.01	<0.01	0.04
Pielou (*J'*)	***0.01***	***<0.01***	0.08	0.10	<0.01
Selected taxa					
No. of *Capitella* spp.	***<0.01***	***0.02***	0.31	<0.01	<0.01
No. of *Ennucula/Nucula*	0.37	0.93	–	–	–
Community structure	***<0.01***	***<0.01***	0.01	<0.01	<0.01

Table 6. Significance probabilities (p) and proportions of variation explained (Prop.) for simple linear regressions of the relationships between sulfide concentration (ln-transformed) as the predictor variable and individual univariate diversity indices as the indicator variable, using data obtained from stations at 2 fish farms and a reference site. Regression analyses for univariate indices were conducted using the DISTLM routine in PERMANOVA+ (using the resemblance matrix of Euclidean distances among samples based on each index alone). For community structure, the same analysis was conducted on the resemblance matrix of Bray-Curtis similarities for square root transformed abundance data. For each sampling station, the abundance data from triplicate grab samples were combined. Probabilities in bold italics are significant ($p<0.05$).

Diversity measure	p	Prop.
No. of individuals (*N*)	0.65	0.01
No. of taxa (*T*)	0.11	0.15
Shannon (*H'*)	***<0.01***	0.42
Margalef (*d*)	0.05	0.22
Pielou (*J'*)	***<0.01***	0.46
No. of *Capitella* spp.	***0.02***	0.31
No. of *Ennucula/Nucula*	0.79	0.01
Community structure	***<0.01***	0.35

Table 7. The 3 most abundant taxa of benthic macrofauna in sediment collected at two salmon farms (sites A & B) and a reference site (C), at 6 stations per site (see Fig. 1). Abundance data from triplicate grab samples were combined at each station. Also shown is the percentage of the total abundance per station due to each taxon, as well as the percentage due to the 3 most abundant taxa combined. Tubificidae excludes *Tubificoides benedii* (only 8 specimens of *T. benedii* were found in total, only at stations B1, B2 & B6).

Station	Sulfide class	Most abundant taxa (% of total number per station)			Combined %
		Most abundant	2nd most abundant	3rd most abundant	
A1	Oxic B	*Capitella* spp. (47%)	*Cossura longocirrata* (6%)	*Eteone longa* (6%)	59
A2	Oxic A	Tubificidae (17%)	*Aricidea jeffrysii* (14%)	*Cossura longocirrata* (10%)	41
A3	Oxic A	Tubificidae (18%)	*Aricidea jeffrysii* (13%)	*Cossura longocirrata* (8%)	39
A4	Oxic A	*Capitella* spp. (22%)	Tubificidae (22%)	*Cossura longocirrata* (6%)	50
A5	Oxic B	*Capitella* spp. (30%)	Tubificidae (11%)	*Pholoe minuta* (8%)	49
A6	Oxic B	*Capitella* spp. (48%)	Acari (10%)	Tubificidae (7%)	64
B1	Hypoxic B	*Capitella* spp. (81%)	Tubificidae (4%)	*Tubificoides benedii* (4%)	89
B2	Oxic A	*Cossura longocirrata* (21%)	*Nucula proxima* (17%)	Tubificidae (13%)	51
B3	Hypoxic A	*Cossura longocirrata* (39%)	Tubificidae (16%)	*Levinsenia gracilis* (10%)	65
B4	Hypoxic A	*Capitella* spp. (86%)	*Mytilus edulis* (9%)	*Eteone longa* (2%)	97
B5	Hypoxic B	*Capitella* spp. (38%)	*Mytilus edulis* (19%)	Tubificidae (11%)	67
B6	Hypoxic A	*Capitella* spp. (83%)	*Mytilus edulis* (9%)	*Eteone longa* (4%)	96
C1	Oxic A	Tubificidae (19%)	*Cossura longocirrata* (18%)	*Tharyx* sp. (10%)	47
C2	Oxic A	Tubificidae (16%)	*Tharyx* sp. (15%)	*Cossura longocirrata* (13%)	44
C3	Oxic A	Tubificidae (28%)	*Cossura longocirrata* (23%)	*Aricidea jeffrysii** (5%)	55
C4	Oxic A	Tubificidae (26%)	*Cossura longocirrata* (25%)	*Tharyx* sp. (9%)	60
C5	Oxic A	*Cossura longocirrata* (22%)	Tubificidae (17%)	*Tharyx* sp. (13%)	52
C6	Oxic A	Tubificidae (19%)	*Tharyx* sp. (14%)	*Cossura longocirrata* (14%)	47

* Tied with *Levinsenia gracilis* for 3rd most abundant taxon at station C3.

Table 8. Contributions of the most important taxa to similarity among stations within sites and to dissimilarity between pairs of sites, based on PRIMER v.6 SIMPER analysis. Abundance data from triplicate grab samples were combined for each of 6 sampling stations per site (farms A & B and reference site C). Analyses were conducted on square root transformed data. Tubificidae excludes *Tubificoides benedii*.

Site(s)	Taxon	Contribution (%)	Cumulative contribution (%)
Site A similarity	*Capitella* spp.	9.3	9.3
(average similarity = 50.2)	Tubificidae	7.3	16.6
	Cossura longocirrata	5.7	22.3
	Aricidea jeffreysii	5.0	27.3
	Nucula proxima	5.0	32.3
Site B similarity	*Capitella* spp.	37.0	37.0
(average similarity = 33.0)	*Mytilus edulis*	16.4	53.4
	Eteone longa	10.6	64.0
	Cerebratulus lacteus	7.2	71.2
	Tubificidae	6.4	77.6
Site C similarity	Tubificidae	12.0	12.0
(average similarity = 63.2)	*Cossura longocirrata*	11.7	23.7
	Tharyx sp.	7.4	31.1
	Aricidea jeffreysii	6.5	37.7
	Levinsenia gracilis	5.1	42.7
Sites A–B dissimilarity	Tubificidae	5.5	5.5
(average dissimilarity = 70.3)	*Capitella* spp.	5.5	10.9
	Aricidea jeffreysii	4.7	15.6
	Cossura longocirrata	4.6	20.3
	Acari	4.0	24.3
Sites A–C dissimilarity	*Capitella* spp.	10.0	10.0
(average dissimilarity = 54.6)	*Tharyx* sp.	4.0	14.0
	Phyllodoce sp.	3.6	17.5
	Pholoe minuta	3.1	20.7
	Tubificidae	3.0	23.7
Sites B–C dissimilarity	*Capitella* spp.	8.0	8.0
(average dissimilarity = 74.5)	*Cossura longocirrata*	7.1	15.1
	Tharyx sp.	6.4	21.5
	Tubificidae	6.3	27.7
	Aricidea jeffreysii	5.1	32.8

Table 9a. Contributions of the most important taxa to similarity among stations within sulfide classes, based on PRIMER v.6 SIMPER analysis. Abundance data from triplicate grab samples were combined for each of 18 sampling stations (6 each at farms A & B and reference site C). The numbers of stations per sulfide class were: Oxic A=10, Oxic B=3, Hypoxic A=3, Hypoxic B=2 (there were no stations classed as Hypoxic C or Anoxic). Analyses were conducted on square root transformed data. Tubificidae excludes *Tubificoides benedii*.

Sulfide class	Taxon	Contribution (%)	Cumulative contribution (%)
Oxic A similarity	Tubificidae	11.7	11.7
(average similarity = 65.2)	*Cossura longocirrata*	10.5	22.1
	Aricidea jeffreysii	6.7	28.8
	Tharyx sp.	5.8	34.6
	Nucula proxima	4.9	39.5
Oxic B similarity	*Capitella* spp.	16.9	16.9
(average similarity = 67.3)	Tubificidae	5.5	22.4
	Cossura longocirrata	5.3	27.7
	Nucula proxima	4.8	32.4
	Aricidea jeffreysii	4.6	37.0
Hypoxic A similarity	*Capitella* spp.	45.4	45.4
(average similarity = 42.9)	*Mytilus edulis*	23.5	68.9
	Eteone longa	12.0	80.9
	Cerebratulus lacteus	8.5	89.4
	Nucula proxima	1.6	91.0
Hypoxic B similarity	*Capitella* spp.	50.1	50.1
(average similarity = 42.5)	Tubificidae	10.8	60.9
	Cossura longocirrata	8.9	69.8
	Eteone longa	8.9	78.6
	Mytilus edulis	8.9	87.5

Table 9b. Contributions of the most important taxa to dissimilarity among stations between pairs of sulfide classes, based on PRIMER v.6 SIMPER analysis. Abundance data from triplicate grab samples were combined for each of 18 sampling stations (6 each at farms A & B and reference site C). The numbers of stations per sulfide class were: Oxic A=10, Oxic B=3, Hypoxic A=3, Hypoxic B=2 (there were no stations classed as Hypoxic C or Anoxic). Analyses were conducted on square root transformed data. Tubificidae excludes *Tubificoides benedii*.

Sulfide classes	Taxon	Contribution (%)	Cumulative contribution (%)
Oxic A–B dissimilarity	*Capitella* spp.	11.8	11.8
(average dissimilarity = 44.5)	*Tharyx* sp.	4.4	16.1
	Tubificidae	3.4	19.5
	Cossura longocirrata	3.2	22.6
	Spiophanes bombyx	2.9	25.6
Oxic A–Hypoxic A dissimilarity	*Capitella* spp.	8.0	8.0
(average dissimilarity = 70.2)	Tubificidae	7.1	15.1
	Cossura longocirrata	6.4	21.6
	Aricidea jeffreysii	5.5	27.1
	Tharyx sp.	5.3	32.4
Oxic A–Hypoxic B dissimilarity	*Cossura longocirrata*	6.9	6.9
(average dissimilarity = 74.0)	Tubificidae	6.4	13.3
	Aricidea jeffreysii	5.4	18.6
	Tharyx sp.	5.3	23.9
	Capitella spp.	5.1	29.0
Oxic B–Hypoxic A dissimilarity	*Capitella* spp.	5.7	5.7
(average dissimilarity = 61.4)	*Cossura longocirrata*	5.2	10.8
	Tubificidae	4.7	15.6
	Acari	4.2	19.8
	Pholoe minuta	4.1	23.9
Oxic B–Hypoxic B dissimilarity	*Capitella* spp.	7.9	7.9
(average dissimilarity = 67.6)	*Pholoe minuta*	4.4	12.3
	Acari	4.1	16.4
	Ninoe nigripes	3.7	20.0
	Cossura longocirrata	3.6	23.6
Hypoxic A–B dissimilarity	*Capitella* spp.	18.2	18.2
(average dissimilarity = 51.5)	*Cossura longocirrata*	9.4	27.7
	Tubificidae	7.9	35.6
	Mytilus edulis	7.9	43.5
	Harpacticoida	5.5	48.9

Table 10. Summary information on SWNB studies reporting data on sulfide concentration and univariate biodiversity indices under salmon farms. N = number of individuals; T = number of taxa; H' = Shannon diversity index; d = Margalef's species richness index; J' = Pielou's evenness index. The diversity indices shown for other studies include only those that were calculated in the present study. The species abundance data reported in Wildish et al. (2002 & 2005) were used to calculate univariate diversity indices.

Source	Sampling date(s)	Sampling method	Sampling unit area (m^2)	Sieve mesh (μm)	Sites	Stations per site	Repli-cates per station	Mean sulfide concentration (μM)	Univariate diversity indices
Present study	Oct-2008	Grab (from boat)	0.072[1]	330	2 active farms 1 reference site	6/6 6	1/1 1	793 / 2 257 92	*N, T, H', d, J',* *Capitella*
Wildish et al. (2001a)	Jun-1999	Wedge core (diver)	0.026	1000	1 active farm 1 reference site	1 1	10 10	12 820 1 876	*T, H', d, J',* *Capitella*
Wildish et al. (2001b)	May-1998	Grab (from boat)	0.100	1000	1 inactive farm (fallow Aug-1997)	6	3	942	*N, T, H'*
Wildish et al. (2002)	Oct-2000 to Sep-2001 (monthly)	Wedge core (diver)	0.079[2]	1000	1 active farm[3] 1 inactive farm (fallow mid-1998)	1 1	1 1	6 – 1 206 39 – 2 245	Species abundance
Wildish et al. (2004b)	Jul-2001 & Sep-2002	Wedge core (diver)	0.026	1000	1 active farm[4] 1 reference site	1 1	5 5	30 000 / 2 500 1 300 / 350	*T, Capitella*
Wildish et al. (2005)	May-1998[5]	Grab (from boat)	0.100	1000	1 new farm (from spring 1998)	6	3	439	Species abundance

[1] three grab samples (0.024 m^2 each) were combined at each of 6 sampling stations at each site.

[2] three wedge core samples (0.026 m^2 each) were combined at each site for each sampling date.

[3] the active farm was sampled at the lease boundary (~70-80 m from the nearest cage); the inactive farm was sampled near the lease centre.

[4] at the farm site, the 5 replicates (on each date) were from 5 different cages; at the reference site, 5 replicate cores were taken within 1 m^2.

[5] biodiversity data were collected on 8 May 1998; sulfide data were collected on 27 May 1998.

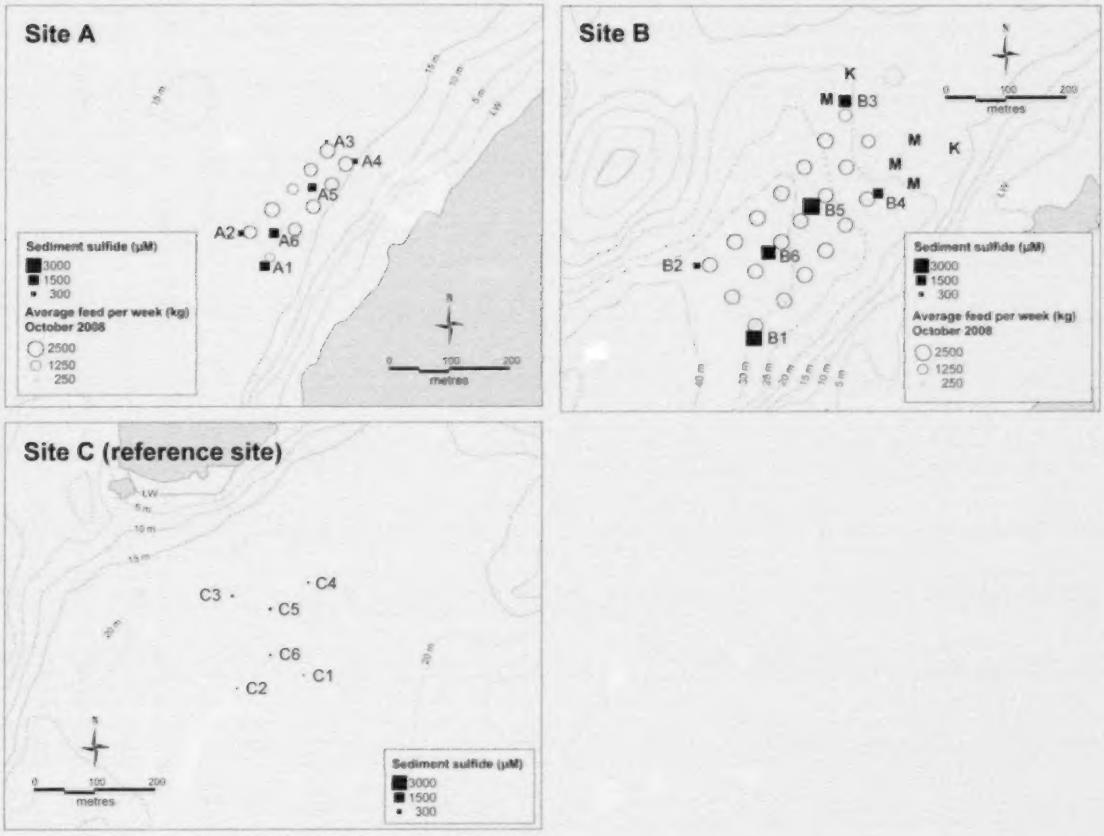

Fig. 1. Maps of 3 study sites in southwestern New Brunswick, showing locations of 6 sampling stations per site. Sites A and B were active salmon farms and site C was a reference site. Three grab samples were collected at each station on 27 October 2008 (sites A and C) and 28 October 2008 (site B). The sizes of the black squares represent mean sediment sulfide concentrations at each station (see figure legends). Circles represent cage locations, with the size of each circle proportional to the average weekly feeding rate at each cage during 28 September to 25 October 2008 (see figure legends). Site B was an integrated multi-trophic aquaculture (IMTA) farm, with mussel (**M**) and kelp (**K**) rafts. Depth contours (metres below lowest normal tide) were derived from Canadian Hydrographic Service field sheets.

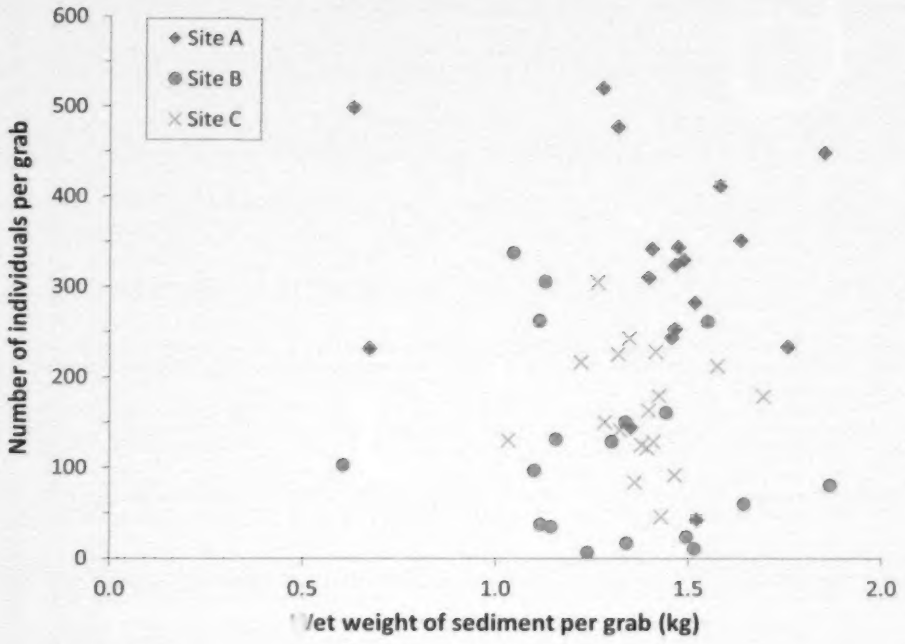

Fig. 2. Number of individuals per grab versus wet weight of sediment per grab at fish farm sites A & B and reference site C.

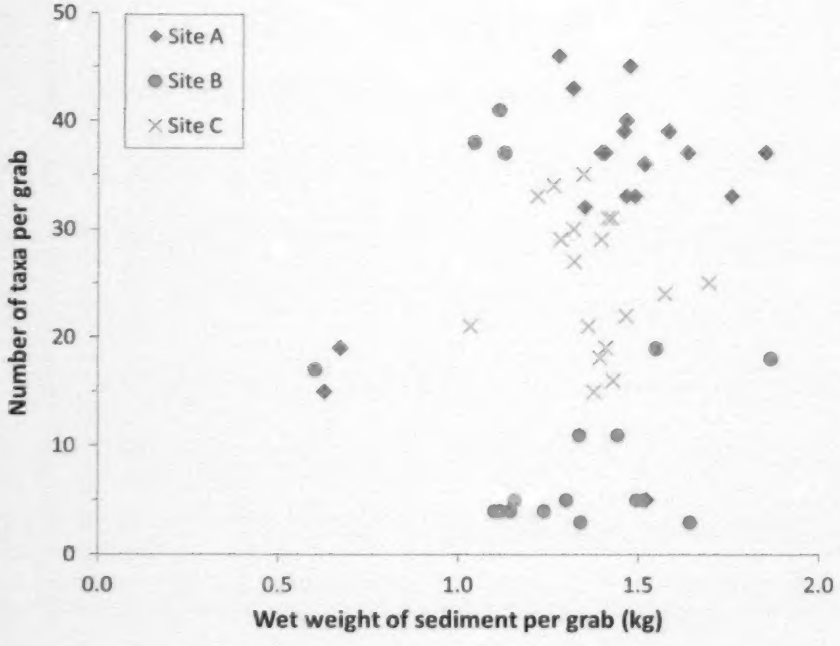

Fig. 3. Number of taxa per grab versus wet weight of sediment per grab at fish farm sites A & B and reference site C.

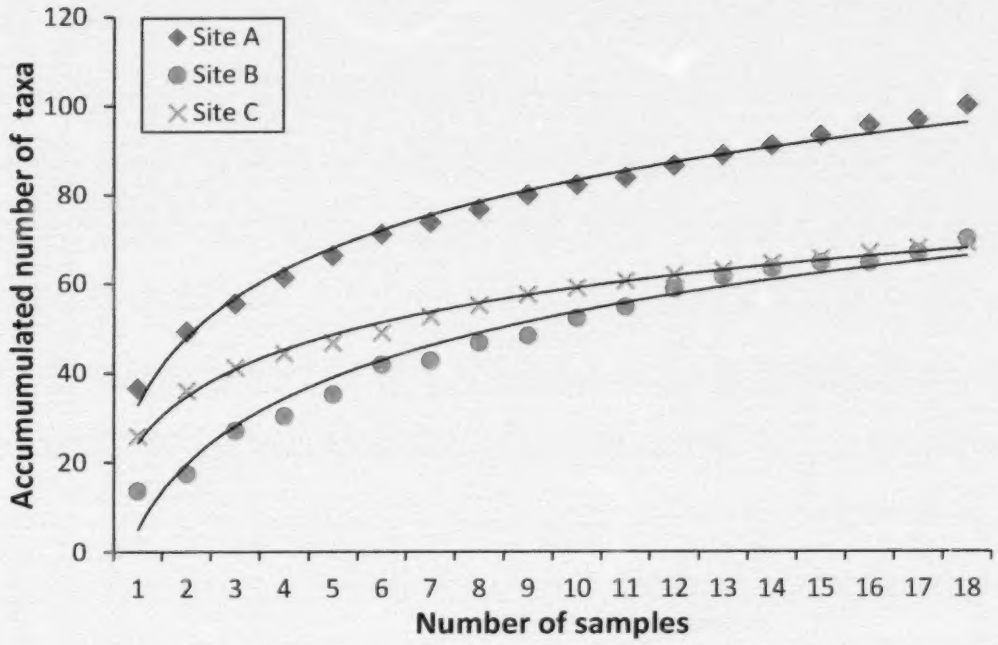

Fig. 4. Accumulated number of observed taxa versus number of grab samples per site, for fish farm sites A & B and reference site C. Accumulation and the fitted curves are based on 10 randomisations of sample number within each site and are estimated by the following logarithmic equations: $T_A = 21.90 \ln(x) + 32.78$ ($R^2 = 0.99$), $T_B = 21.20 \ln(x) + 4.89$ ($R^2 = 0.96$), $T_C = 15.08 \ln(x) + 24.31$ ($R^2 = 0.99$). T = number of taxa; x = number of samples.

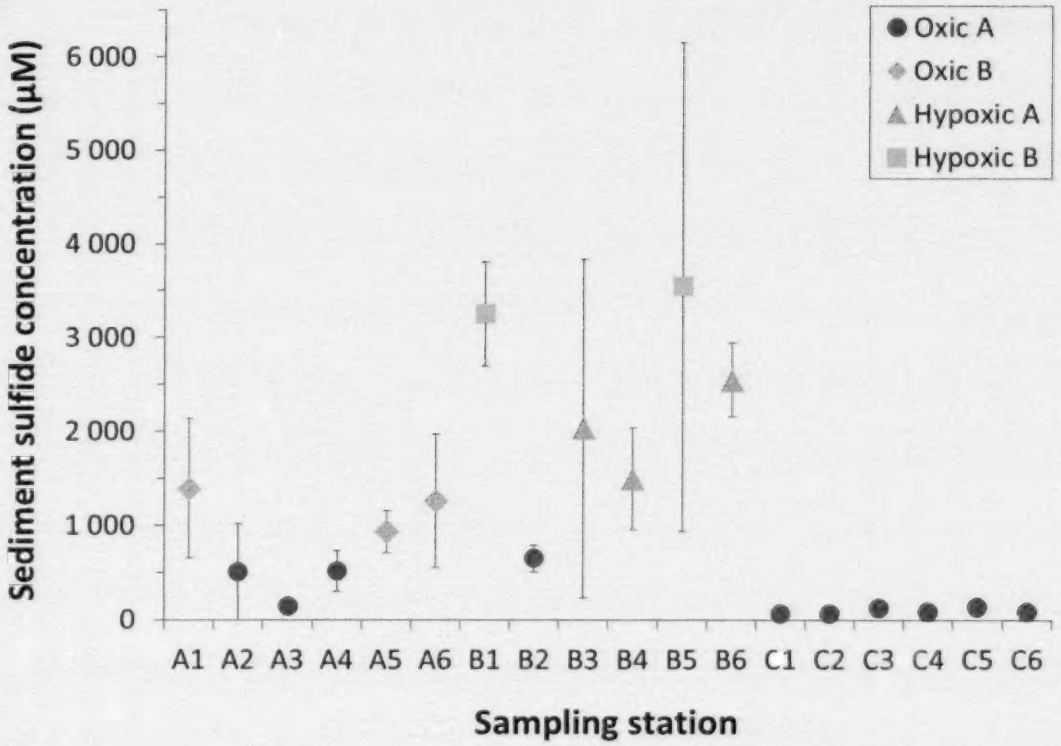

Fig. 5a. Sediment sulfide concentration (total free S^{2-}, µM) at sampling stations at two salmon farms (sites A & B) and a reference site (C). Six stations were sampled at each site (see Fig. 1), with 9 sulfide measurements per station (3 grab samples per station and 3 sulfide measurements per grab sample). Mean sulfide concentration ± standard deviation (SD) at each sampling station.

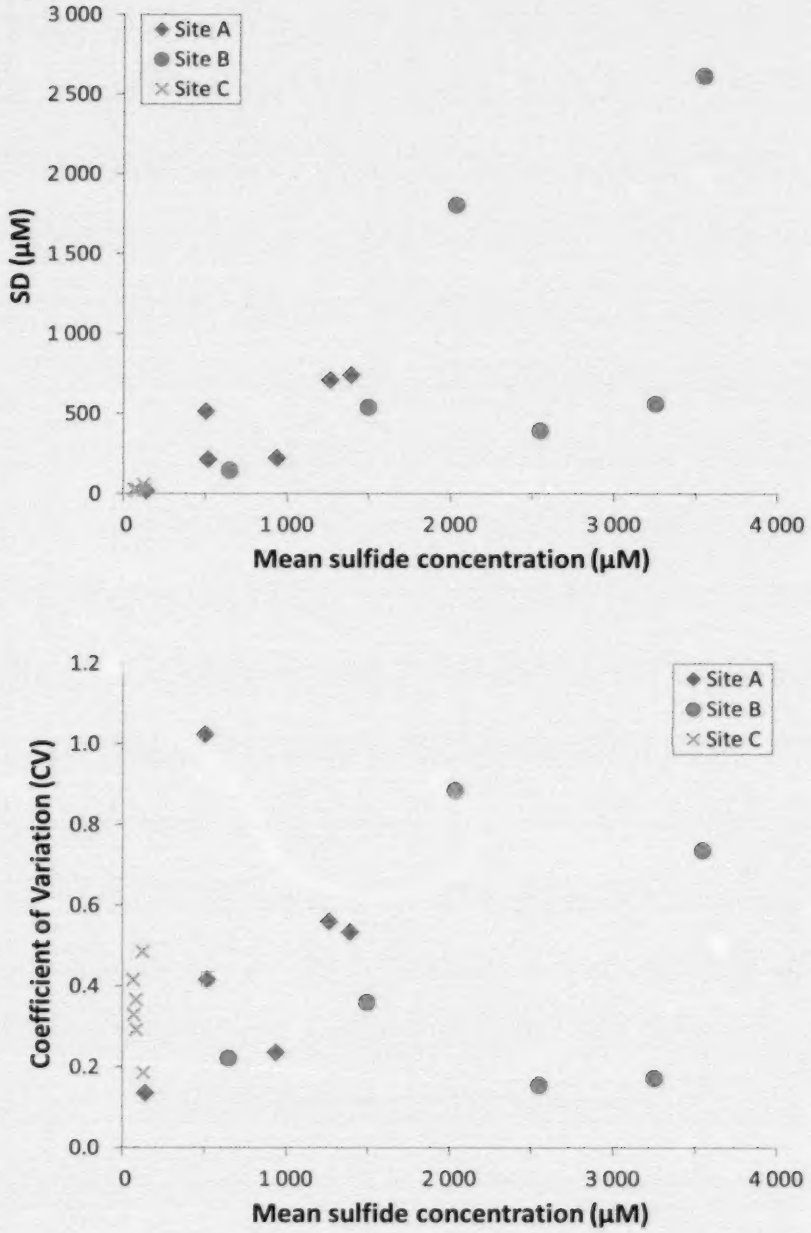

Fig. 5b. Top: mean sulfide concentration vs. standard deviation (SD). Bottom: mean sulfide concentration vs. coefficient of variation (CV = SD/mean).

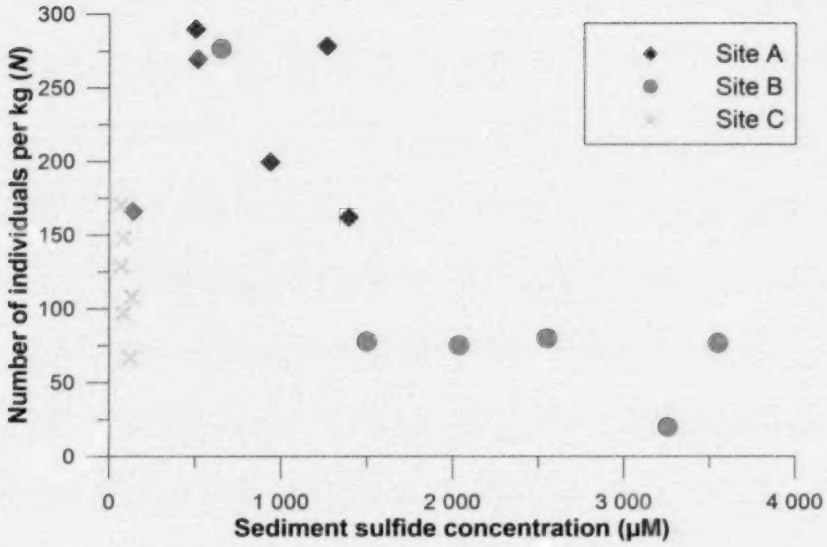

Fig. 6. Number of benthic macrofauna individuals per kg (wet weight) of sediment (*N*) vs. sediment sulfide concentration at sampling stations under two salmon farms (sites A & B) and a reference site (C). Abundance data were combined from triplicate grabs at each of 6 stations per site (see Fig. 1).

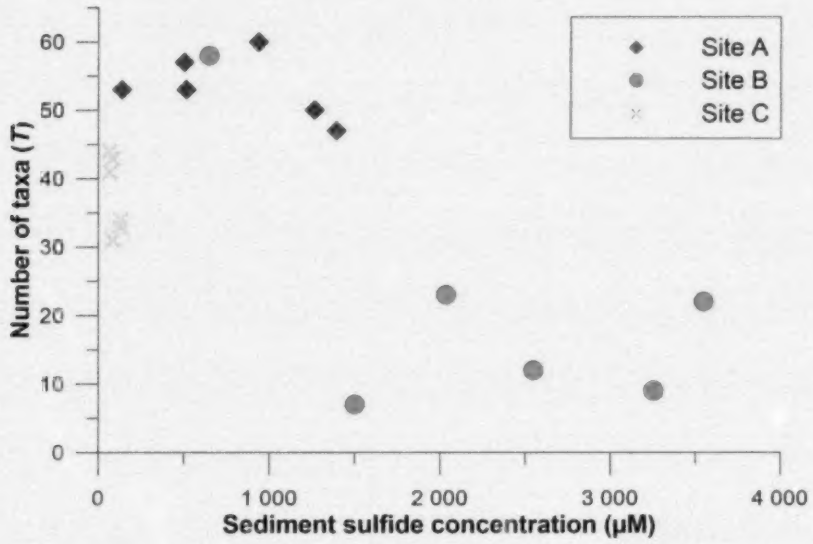

Fig. 7. Number of benthic macrofauna taxa (*T*) vs. sediment sulfide concentration at sampling stations under two salmon farms (sites A & B) and a reference site (C). Abundance data were combined from triplicate grabs at each of 6 stations per site (see Fig. 1). Most individuals were identified to the species level.

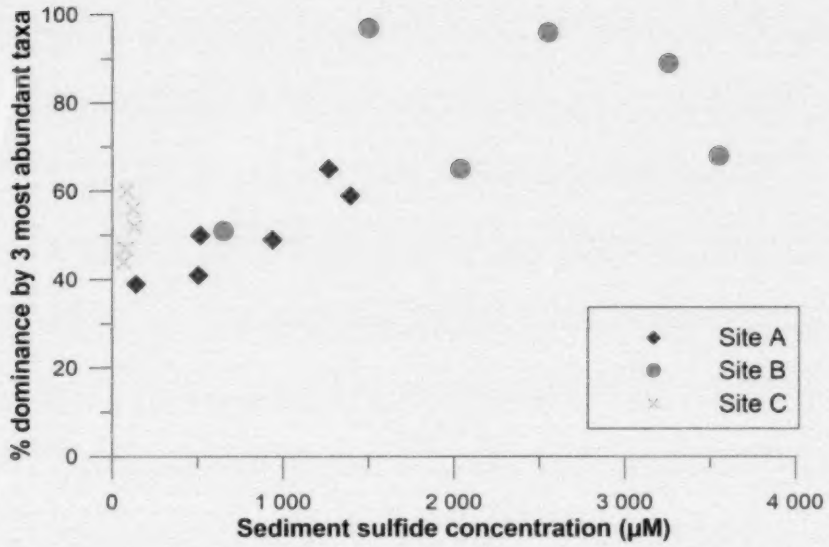

Fig. 8. Percent dominance by the 3 most common taxa (combined) vs. sediment sulfide concentration at stations under two salmon farms (sites A & B) and a reference site (C). Abundance data were combined from triplicate grabs at each of 6 stations per site (see Fig. 1).

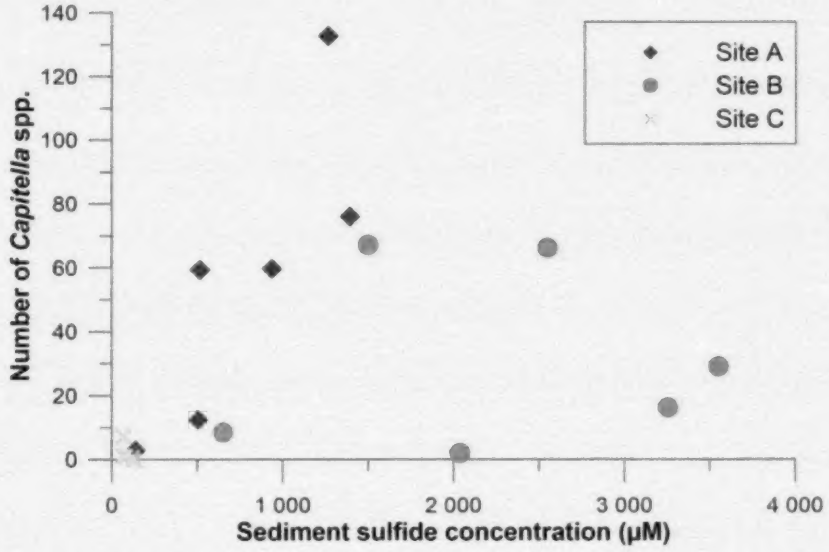

Fig. 9. Number of *Capitella* spp. per kg (wet weight) of sediment vs. sediment sulfide concentration at stations under two salmon farms (sites A & B) and a reference site (C). Abundance data were combined from triplicate grabs at each of 6 stations per site (see Fig. 1).

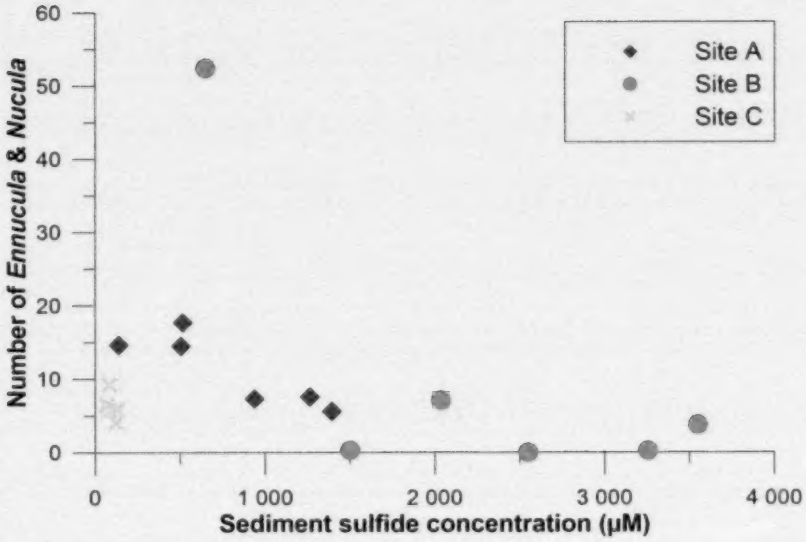

Fig. 10. Number of *Ennucula delphinodonta* and *Nucula proxima* (combined) per kg (wet weight) of sediment vs. sediment sulfide concentration at stations under two salmon farms (sites A & B) and a reference site (C). Abundance data were combined from triplicate grabs at each of 6 stations per site (see Fig. 1).

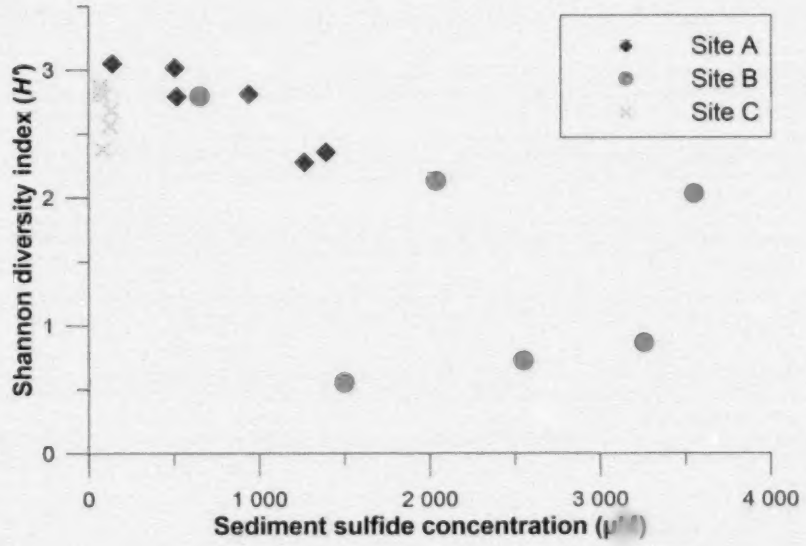

Fig. 11. Shannon diversity index (*H'*) vs. sediment sulfide concentration for benthic macrofauna at stations under two salmon farms (sites A & B) and a reference site (C). Abundance data were combined from triplicate grabs for each of 6 stations per site (see Fig. 1).

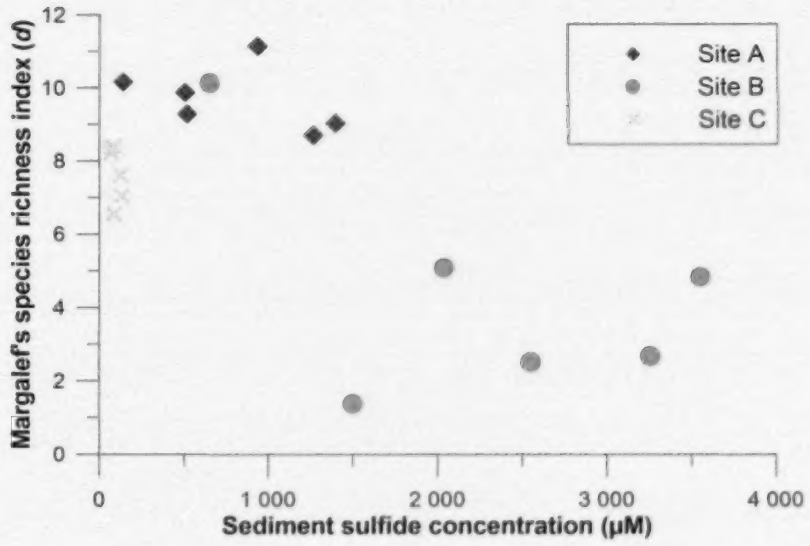

Fig. 12. Margalef's species richness index (d) vs. sediment sulfide concentration for benthic macrofauna at stations under two salmon farms (sites A & B) and a reference site (C). Abundance data were combined from triplicate grabs at each of 6 stations per site (see Fig. 1).

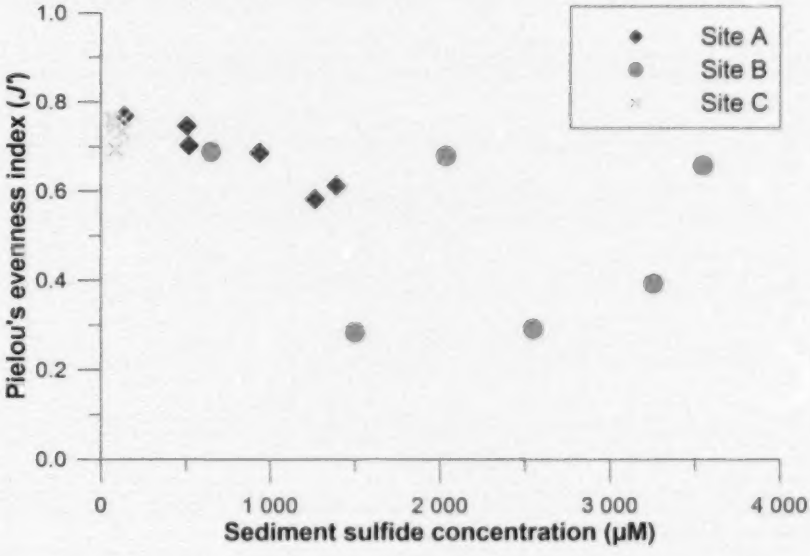

Fig. 13. Pielou's evenness index (J') vs. sediment sulfide concentration for benthic macrofauna at stations under two salmon farms (sites A & B) and a reference site (C). Abundance data were combined from triplicate grabs at each of 6 stations per site (see Fig. 1).

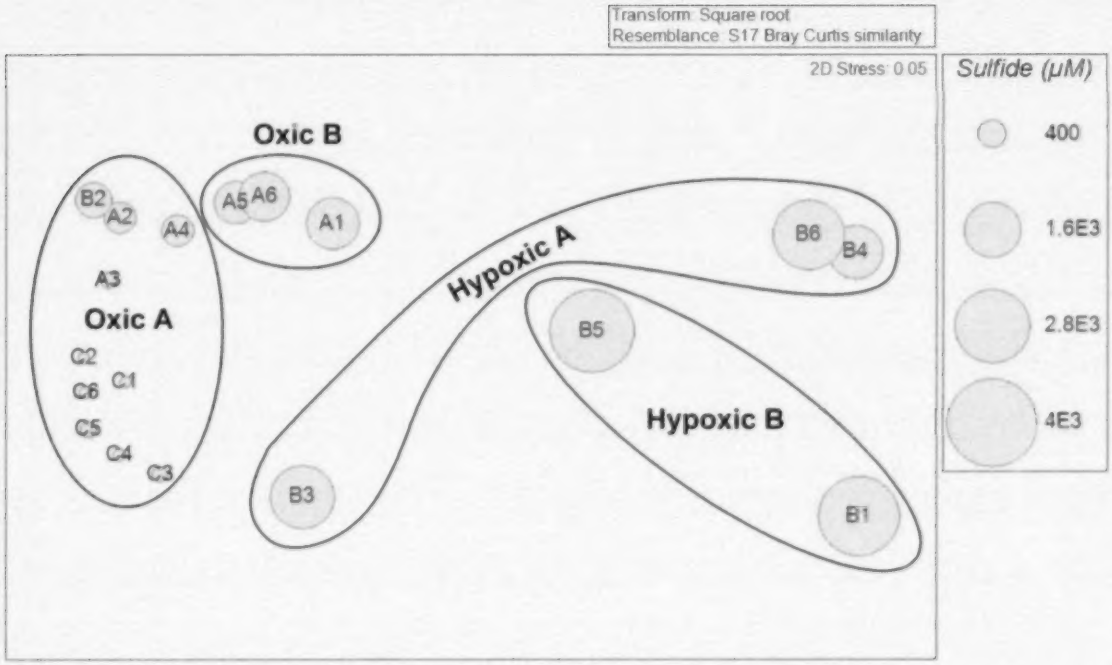

Fig. 14. Multi-dimensional scaling (MDS) bubble plot of benthic macrofaunal abundance data (square-root transformed) at sampling stations under two salmon farms (sites A & B) and a reference site (C). MDS plots were produced using PRIMER v6 software. Abundance data were combined from triplicate grabs at each of 6 stations per site (see Fig. 1). Circle sizes are in proportion to the mean sediment sulfide concentration at each station (see legend). Clusters of stations within sulfide classes from Oxic A to Hypoxic B are shown; there were no stations classified as Hypoxic C or Anoxic.

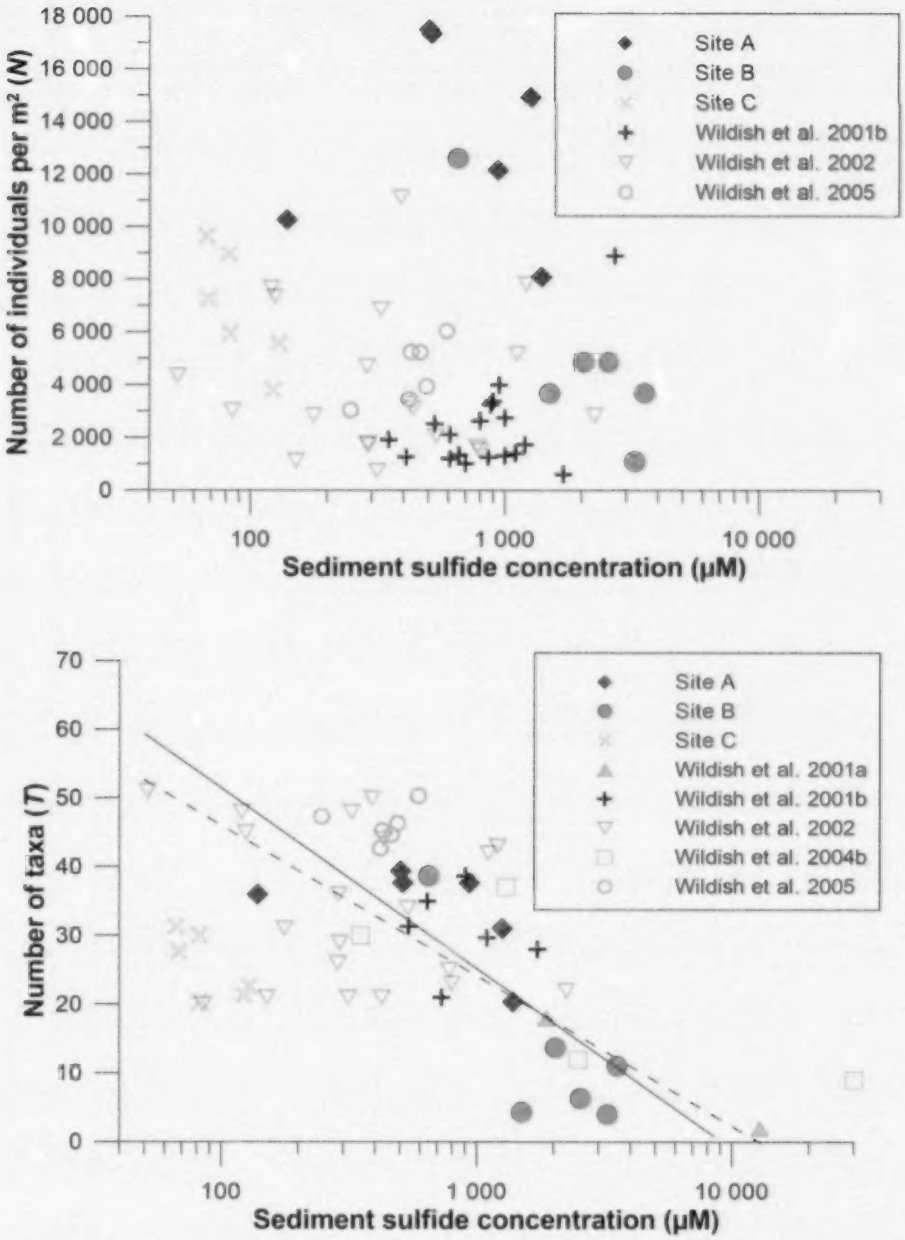

Fig. 15. Univariate biodiversity indices vs. sediment sulfide concentration from the present study (fish farm sites A & B and reference site C) and previous studies in SWNB. Top: Number of individuals per m^2 (*N*). Bottom: Number of taxa (*T*). Data for the present study were collected in October 2008 and for the previous studies between 1998 and 2002. Not shown are two points from Wildish et al. (2002) where the mean sulfide concentration was <50 µM (the October 2000 sampling event at each of the two sites in that study). The lines in the lower graph represent empirical relationships from Hargrave (2010).

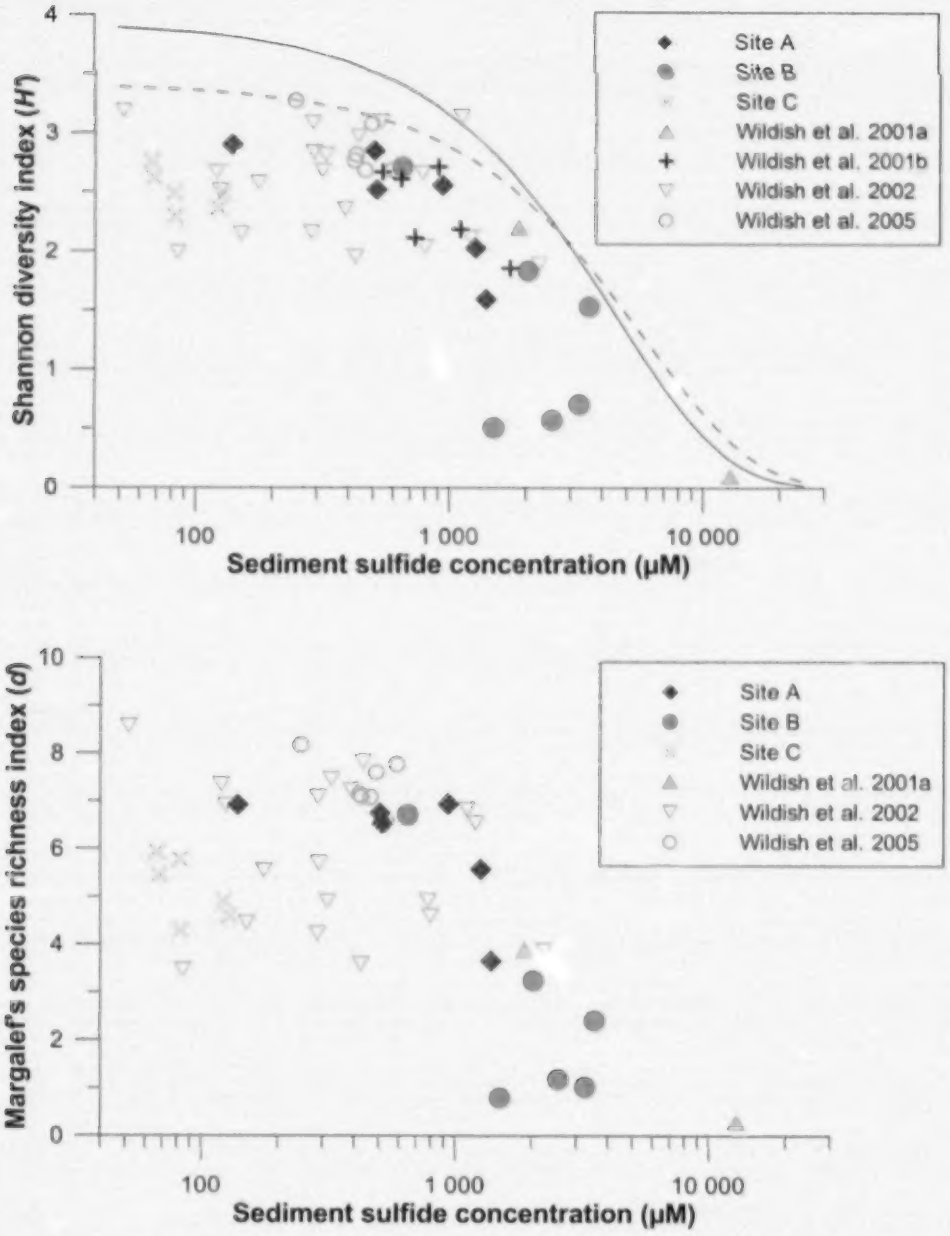

Fig. 15 (cont'd). Top: Shannon diversity index (H'). Bottom: Margalef's species richness index (d). The lines in the upper graph represent empirical relationships from Hargrave (2010).

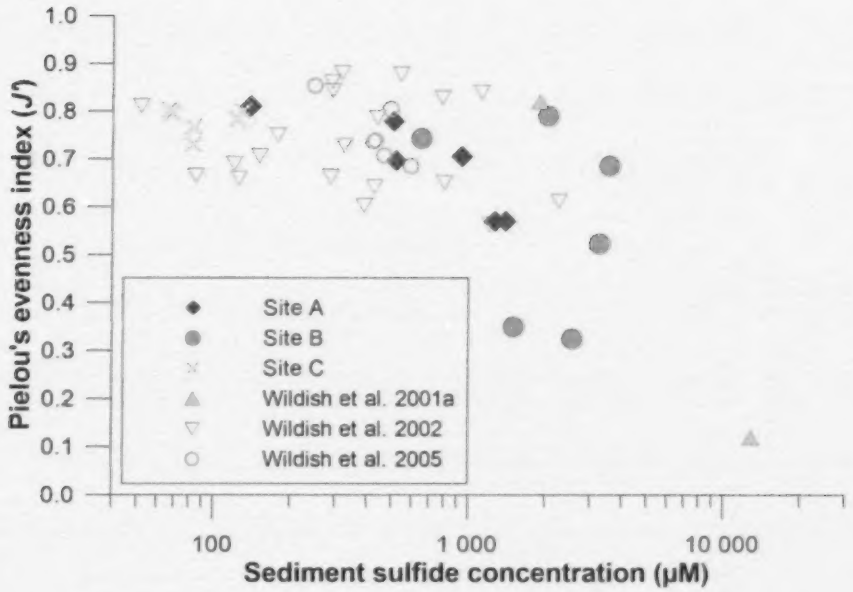

Fig. 15 (concluded). Pielou's evenness index (*J'*).

SULFIDE CONCENTRATION DATA

Table A1. Sediment sulfide concentration (total free S^{2-}, µM) in grab samples collected at 2 salmon farms (sites A & B) and a reference site (C). Triplicate samples (grabs 1, 2 & 3) were collected at each of 6 stations at each site (see Fig. 1). From each grab sample, triplicate subsamples (a, b & c) were taken for sulfide analyses (for a total of 9 sulfide measurements per sampling station).

	Grab 1					Grab 2					Grab 3					Station	
Station	a	b	c	Mean	SD	a	b	c	Mean	SD	a	b	c	Mean	SD	Mean	SD
Site A																	
A1	1 270	2 720	980	1 657	932	718	557	721	665	94	1 540	1 850	2 180	1 857	320	1 393	742
A2	1 710	968	506	1 061	607	229	178	189	199	37	257	246	264	256	9	505	516
A3	131	109	140	127	16	140	179	137	152	23	132	151	132	138	11	139	19
A4	363	429	293	362	68	488	373	361	407	70	707	936	694	779	136	516	215
A5	1 035	790	672	832	185	787	1 370	729	962	355	1 110	920	1 026	1 019	95	938	222
A6	1 650	1 169	1 430	1 416	241	2 230	1 920	1 760	1 970	239	487	479	267	411	125	1 266	708
Site																**793**	**645**
Site B																	
B1	2 890	3 190	3 310	3 130	216	2 490	2 780	2 980	2 750	246	3 790	4 290	3 580	3 887	365	3 256	558
B2	614	616	790	673	101	433	431	720	528	166	826	746	673	748	77	650	143
B3	298	363	427	363	65	1 576	1 400	1 200	1 392	188	4 480	4 440	4 150	4 357	180	2 037	1 801
B4	1 160	1 280	903	1 114	193	1 560	2 030	2 670	2 087	557	1 360	1 350	1 190	1 300	95	1 500	538
B5	6 300	7 360	6 720	6 793	534	1 220	700	858	926	267	2 510	3 450	2 830	2 930	478	3 550	2 611
B6	2 270	2 240	2 320	2 277	40	3 270	2 810	2 690	2 923	306	2 660	2 730	1 960	2 450	426	2 550	391
Site																**2 257**	**1 627**
Site C																	
C1	80	59	73	71	11	36	33	62	44	16	87	95	88	90	4	68	22
C2	95	21	68	61	37	92	92	61	82	18	91	37	44	57	29	67	28
C3	76	59	108	81	25	88	114	65	89	25	187	205	203	198	10	123	60
C4	130	97	120	116	17	62	31	70	54	21	89	76	69	78	10	83	30
C5	126	139	168	144	22	110	124	91	108	17	158	112	131	134	23	129	24
C6	78	87	123	96	24	102	74	92	89	14	35	72	77	61	23	82	24
Site																**92**	**41**

LIST OF TAXA

List of benthic macrofaunal taxa from grab samples at 2 fish farm sites (A & B) and a reference site (C) in southwestern New Brunswick, Bay of Fundy, with abundance per site. Species names and classifications are from the World Register of Marine Species (www.marinespecies.org, accessed July 2016); except where no designation was provided for a given level, the designation from the Integrated Taxonomic Information System on-line database (www.itis.gov; accessed July 2016) was used [indicated by square brackets]. Subcategories (e.g., subphylum, infraclass, subclass, superorder) are not shown. The abundance data (number of individuals) are totals per site (from 18 grabs per site). The total wet weights of all grabs per site were: 25.25 kg at site A, 23.10 kg at site B, and 24.72 kg at site C.

					Abundance per site			
Phylum	**Class**	**Order**	**Family**	**Lowest taxon**	**Site A**	**Site B**	**Site C**	**Total**
Porifera	Demospongiae	Suberitida	Suberitidae	*Suberites ficus*	27	1	0	28
Cnidaria	Anthozoa	Actiniaria	Actiniidae	*Urticina felina*	0	1	0	1
Cnidaria	Anthozoa	Spirularia	Cerianthidae	*Pachycerianthus borealis*	1	3	1	5
Nemertea	Anopla	[Heteronemertea]	Lineidae	*Cerebratulus lacteus*	22	28	4	54
Nemertea	Anopla	[Heteronemertea]	Lineidae	*Micrura* sp.	1	0	1	2
Nemertea	Enopla	Monostilifera	Amphiporidae	*Amphiporus* sp.	1	0	2	3
Nemertea	Enopla	Monostilifera	Tetrastemmatidae	*Tetrastemma* sp.	7	3	22	32
Nemertea	unknown	unknown	unknown	unidentified nemertine	1	0	2	3
Mollusca	Bivalvia	Adapedonta	Hiatellidae	*Hiatella arctica*	48	2	0	50
Mollusca	Bivalvia	Carditida	Astartidae	*Astarte undata*	19	6	4	29
Mollusca	Bivalvia	Carditida	Carditidae	*Cyclocardia borealis*	4	0	0	4
Mollusca	Bivalvia	Lucinida	Thyasiridae	*Thyasira gouldii*	49	1	4	54
Mollusca	Bivalvia	Myida	Myidae	*Mya arenaria*	8	3	4	15
Mollusca	Bivalvia	Myida	Myidae	*Mya truncata*	1	0	0	1
Mollusca	Bivalvia	Mytilida	Mytilidae	*Crenella glandula*	20	1	40	61
Mollusca	Bivalvia	Mytilida	Mytilidae	*Musculus niger*	3	0	1	4
Mollusca	Bivalvia	Mytilida	Mytilidae	*Mytilus edulis*	145	165	67	377
Mollusca	Bivalvia	Nuculanida	Nuculanidae	*Nuculana* sp.	2	0	1	3
Mollusca	Bivalvia	Nuculanida	Yoldiidae	*Yoldia myalis*	20	3	27	50
Mollusca	Bivalvia	Nuculida	Nuculidae	*Ennucula delphinodonta*	39	31	63	133
Mollusca	Bivalvia	Nuculida	Nuculidae	*Nucula proxima*	252	189	95	536
Mollusca	Bivalvia	Pectinida	Anomiidae	*Anomia simplex*	2	0	1	3
Mollusca	Bivalvia	Solemyida	Solemyidae	*Solemya velum*	1	1	0	2
Mollusca	Bivalvia	Venerida	Arcticidae	*Arctica islandica*	6	1	1	8
Mollusca	Bivalvia	Venerida	Veneridae	*Pitar morrhuanus*	3	0	0	3
Mollusca	Bivalvia	[Pholadomyoida]	Lyonsiidae	*Lyonsia hyalina*	8	1	30	39
Mollusca	Bivalvia	[Pholadomyoida]	Periplomatidae	*Periploma leanum*	44	2	17	63

Phylum	Class	Order	Family	Lowest taxon	Abundance per site			
					Site A	Site B	Site C	Total
Mollusca	Gastropoda	Cephalaspidea	Cylichnidae	*Cylichna alba*	4	1	6	11
Mollusca	Gastropoda	Cephalaspidea	Retusidae	*Retusa obtusa*	0	1	0	1
Mollusca	Gastropoda	Littorinimorpha	Littorinidae	*Lacuna vincta*	0	3	0	3
Mollusca	Gastropoda	Littorinimorpha	Rissoidae	*Frigidoalvania* sp.	8	4	50	62
Mollusca	Gastropoda	Littorinimorpha	Velutinidae	*Velutina* sp.	1	1	0	2
Mollusca	Gastropoda	Neogastropoda	Buccinidae	*Colus pygmaeus*	0	1	1	2
Mollusca	Gastropoda	Neogastropoda	Columbellidae	*Astyris* sp.	0	0	1	1
Mollusca	Gastropoda	Neogastropoda	Mangeliidae	*Oenopota elegans*	0	0	1	1
Mollusca	Gastropoda	Neogastropoda	Nassaridae	*Tritia trivittata*	4	1	0	5
Mollusca	Gastropoda	Nudibranchia	Onchidoridiidae	*Acanthodoris pilosa*	1	0	0	1
Mollusca	Gastropoda	[Patellogastropoda]	Lottiidae	*Testudinalia testudinalis*	1	2	0	3
Mollusca	Gastropoda	[Archaeogastropoda]	Margaritidae	*Margarites helicina*	1	0	0	1
Sipuncula	Sipunculidea	Golfingiida	Phascolionidae	*Phascolion strombus*	2	4	1	7
Annelida	Polychaeta	Eunicida	Lumbrineridae	*Lumbrineris fragilis*	26	13	11	50
Annelida	Polychaeta	Eunicida	Lumbrineridae	*Lumbrineris tenuis*	74	14	21	109
Annelida	Polychaeta	Eunicida	Lumbrineridae	*Ninoe nigripes*	205	49	74	328
Annelida	Polychaeta	Phyllodocida	Glyceridae	*Glycera dibranchiata*	4	0	0	4
Annelida	Polychaeta	Phyllodocida	Hesionidae	*Microphthalmus aberrans*	0	2	0	2
Annelida	Polychaeta	Phyllodocida	Nephtyidae	*Bipalponephtys neotena*	4	1	0	5
Annelida	Polychaeta	Phyllodocida	Nephtyidae	*Nephtys ciliata*	0	0	1	1
Annelida	Polychaeta	Phyllodocida	Nephtyidae	*Nephtys incisa*	14	3	7	24
Annelida	Polychaeta	Phyllodocida	Nereidiidae	*Neanthes diversicolor*	3	0	0	3
Annelida	Polychaeta	Phyllodocida	Nereidiidae	*Nereis zonata*	1	0	0	1
Annelida	Polychaeta	Phyllodocida	Nereidiidae	*Nereis* sp. juvenile	0	1	0	1
Annelida	Polychaeta	Phyllodocida	Pholoidae	*Pholoe minuta*	261	62	47	370
Annelida	Polychaeta	Phyllodocida	Phyllodocidae	*Eteone longa*	126	65	47	238
Annelida	Polychaeta	Phyllodocida	Phyllodocidae	*Phyllodoce* sp.	186	22	6	214
Annelida	Polychaeta	Phyllodocida	Polynoidae	*Harmothoe extenuata*	4	1	0	5
Annelida	Polychaeta	Phyllodocida	Polynoidae	*Harmothoe imbricata*	13	1	0	14
Annelida	Polychaeta	Phyllodocida	Sphaerodoridae	*Sphaerodoropsis minuta*	0	1	0	1
Annelida	Polychaeta	Phyllodocida	Syllidae	*Eusyllis* sp.	1	0	0	1
Annelida	Polychaeta	Phyllodocida	Syllidae	*Exogone* sp.	10	1	0	11
Annelida	Polychaeta	Phyllodocida	Syllidae	*Syllis comuta*	2	1	2	5
Annelida	Polychaeta	Phyllodocida	Syllidae	other Syllidae	4	1	27	32

Phylum	Class	Order	Family	Lowest taxon	Abundance per site			Total
					Site A	Site B	Site C	
Annelida	Polychaeta	Sabellida	Oweniidae	*Galathowenia oculata*	3	1	12	16
Annelida	Polychaeta	Sabellida	Sabellidae	Sabellidae	4	0	0	4
Annelida	Polychaeta	[Scolecida]	Capitellidae	*Capitella* spp.	1389	719	48	2156
Annelida	Polychaeta	[Scolecida]	Capitellidae	*Heteromastus filiformis*	0	0	2	2
Annelida	Polychaeta	[Scolecida]	Capitellidae	*Mediomastus ambiseta*	8	1	2	11
Annelida	Polychaeta	[Scolecida]	Cossuridae	*Cossura longocirrata*	368	332	532	1232
Annelida	Polychaeta	[Scolecida]	Maldanidae	*Maldane sarsi*	0	0	3	3
Annelida	Polychaeta	[Scolecida]	Maldanidae	*Praxillella praetermissa*	1	0	0	1
Annelida	Polychaeta	[Scolecida]	Maldanidae	*Rhodine loveni*	31	1	44	76
Annelida	Polychaeta	[Scolecida]	Opheliidae	*Ophelina aulogaster*	28	36	0	64
Annelida	Polychaeta	[Scolecida]	Paraonidae	*Aricidea jeffreysii*	407	32	262	701
Annelida	Polychaeta	[Scolecida]	Paraonidae	*Aricidea quadrilobata*	12	0	18	30
Annelida	Polychaeta	[Scolecida]	Paraonidae	*Levinsenia gracilis*	168	41	110	319
Annelida	Polychaeta	[Scolecida]	Scalibregmatidae	*Scalibregma inflatum*	0	2	0	2
Annelida	Polychaeta	Spionida	Apistobranchidae	*Apistobranchus tulbergi*	0	0	2	2
Annelida	Polychaeta	Spionida	Spionidae	*Polydora sp.*	2	0	0	2
Annelida	Polychaeta	Spionida	Spionidae	*Spiophanes bombyx*	62	1	0	63
Annelida	Polychaeta	Spionida	Spionidae	other Spionidae	45	16	1	62
Annelida	Polychaeta	Terebellida	Ampharetidae	*Ampharete acutifrons*	80	12	47	139
Annelida	Polychaeta	Terebellida	Ampharetidae	*Ampharete oculata*	4	0	0	4
Annelida	Polychaeta	Terebellida	Ampharetidae	*Melinna cristata*	5	0	2	7
Annelida	Polychaeta	Terebellida	Ampharetidae	*Samythella elongata*	1	0	0	1
Annelida	Polychaeta	Terebellida	Cirratulidae	*Tharyx* sp.	65	21	347	433
Annelida	Polychaeta	Terebellida	Flabelligeridae	*Brada granosa*	1	0	0	1
Annelida	Polychaeta	Terebellida	Flabelligeridae	*Brada villosa*	1	1	1	3
Annelida	Polychaeta	Terebellida	Flabelligeridae	*Pherusa* sp.	0	0	1	1
Annelida	Polychaeta	Terebellida	Pectinariidae	*Cistenides granulata*	1	0	0	1
Annelida	Polychaeta	Terebellida	Sternaspidae	*Sternaspis scutata*	1	0	0	1
Annelida	Polychaeta	Terebellida	Terebellidae	*Neoamphitrite figulus*	6	0	0	6
Annelida	Polychaeta	Terebellida	Terebellidae	*Pista maculata*	1	0	0	1
Annelida	Polychaeta	Terebellida	Trichobranchidae	*Terebellides stroemi*	69	2	81	152
Annelida	Clitellata (subclass Oligochaeta)	Haplotaxida	Tubificidae	*Tubificoides benedii*	0	8	0	8
Annelida	Clitellata (subclass Oligochaeta)	Haplotaxida	Tubificidae	other Tubificidae	804	211	590	1605

Phylum	Class	Order	Family	Lowest taxon	Abundance per site			Total
					Site A	Site B	Site C	
Cephalorhyncha	Kinorhyncha	Homalorhagida	Pycnophyidae	*Pycnophyes frequens*	25	5	0	30
Cephalorhyncha	Priapulida	not designated	Priapulidae	*Priapulus caudatus*	0	0	1	1
Arthropoda	Arachnida (subclass Acari)	unknown	unknown	unidentified Acari	273	7	96	376
Arthropoda	Cephalocarida	Brachypoda	Hutchinsoniellidae	*Hutchinsoniella* sp.	0	0	5	5
Arthropoda	Malacostraca	Amphipoda	Ampeliscidae	*Ampelisca abdita*	63	0	0	63
Arthropoda	Malacostraca	Amphipoda	Caprellidae	*Aeginina longicornis*	0	0	11	11
Arthropoda	Malacostraca	Amphipoda	Cheirocratidae	*Casco bigelowi*	3	0	4	7
Arthropoda	Malacostraca	Amphipoda	Corophiidae	*Corophium* sp.	8	1	0	9
Arthropoda	Malacostraca	Amphipoda	Corophiidae	*Leptocheirus pinguis*	5	0	0	5
Arthropoda	Malacostraca	Amphipoda	Maeridae	*Maera danae*	2	0	0	2
Arthropoda	Malacostraca	Amphipoda	Photidae	*Photis* sp.	8	0	2	10
Arthropoda	Malacostraca	Amphipoda	Phoxocephalidae	*Harpinia propinqua*	1	0	0	1
Arthropoda	Malacostraca	Amphipoda	Phoxocephalidae	*Phoxocephalus holboli*	0	3	0	3
Arthropoda	Malacostraca	Amphipoda	Pontogeneiidae	*Pontogeneia inermis*	1	0	0	1
Arthropoda	Malacostraca	Amphipoda	Unciolidae	*Unciola irrorata*	1	0	0	1
Arthropoda	Malacostraca	Cumacea	Diastylidae	*Diastylis quadrispinosa*	8	2	1	11
Arthropoda	Malacostraca	Cumacea	Diastylidae	*Diastylis sculpta*	3	0	2	5
Arthropoda	Malacostraca	Cumacea	Leuconidae	*Eudorella pusilla*	1	0	1	2
Arthropoda	Malacostraca	Isopoda	Idoteidae	*Edotia montosa*	4	5	5	14
Arthropoda	[Maxillopoda]	Harpacticoida	unknown	unidentified Harpacticoida	68	47	19	134
Arthropoda	Ostracoda	unknown	unknown	unidentified Ostracoda	37	8	21	66
Arthropoda	Pycnogonida	Pantopoda	Ammotheidae	*Achelia spinosa*	1	0	0	1
Arthropoda	Pycnogonida	Pantopoda	Nymphonidae	*Nymphon* sp.	1	0	0	1
Arthropoda	Pycnogonida	Pantopoda	Phoxichilidiidae	*Phoxichilidium femoratum*	1	0	0	1
Echinodermata	Echinoidea	Camarodonta	Strongylocentrotidae	*Strongylocentrotus droebachiensis*	0	1	0	1
Echinodermata	Holothuroidea	Dendrochirotida	Cucumariidae	*Cucumaria frondosa*	1	0	0	1
Echinodermata	Ophiuroidea	Ophiurida	Amphiuridae	*Amphipholis squamata*	0	0	1	1
Hemichordata	Enteropneusta	Enteropneusta	Harrimaniidae	*Saccoglossus kowalewskii*	0	0	2	2
Chordata	Ascidiacea	Phlebobranchia	Cionidae	*Ciona intestinalis*	1	1	0	2

EFFECT OF SAMPLING UNIT SIZE ON DIVERSITY MEASURES

Introduction

In this analysis, we examined the effect of the sampling unit size on the various diversity measures, using the grab sample data collected in the study described in the main report. We derived a range of sampling unit sizes through consolidation of data from individual grab samples, and then calculated the diversity measures using the abundance data for the different sampling units.

Methods

Different sampling units were derived by summing the abundance data from the individual triplicate grab samples collected at 6 stations at each of 3 sites: 2 active salmon farms (sites A & B) and a reference site (C). The sampling units were: grab samples (abundance data per individual grab); stations (sums of abundance data from 3 grabs per station; 6 stations per site); station pairs (sums of abundance data from 6 grabs per station pair; 3 station pairs per site); and entire sites (sums of abundance data from 18 grabs per site). The three station pairs per site were: the two stations at the southern corners (stations 1 & 2 at each site), the two stations at the northern corners (stations 3 & 4), and the two stations in the interior of each site (stations 5 & 6) (see Fig. 1 of the main report).

The sampling unit sizes were based on the surface areas of the grab samples (using the grab dimensions). The sizes of the different sampling units were calculated as the sums of the surface areas of the individual grabs that were consolidated into each sampling unit: 0.024 m^2 per grab sample (15 × 16 cm); 0.072 m^2 per station (3 grab samples combined); 0.144 m^2 per station pair (6 grab samples combined); and 0.432 m^2 per site (18 grab samples combined). The 3 grab samples at each station were from approximately the same location; stations and station pairs were more widely separated (see Fig. 1 of main report).

The total number of individuals (as well as the numbers of *Capitella* spp. and *Ennucula/Nucula*), univariate diversity indices, and MDS plots were derived for each of the derived sampling units (see the main report for methods). Because MDS plots require >3 sampling units, this type of plot could not be created for the "site" sampling unit (since there were only 3 sites).

Sulfide variability was also calculated for each sampling unit at each site. The number of sulfide measurements per sampling unit was: 3 per grab sample (18 sampling units per site); 9 per station (6 sampling units per site); 18 per station pair (3 sampling units per site); and 54 per site (1 sampling unit per site). The mean, standard deviation (SD), and coefficient of variation (CV = SD/mean) were calculated for all of the sulfide measurements within each sampling unit, for each size of sampling unit.

The probablities of significant differences between sites for sulfide concentration, the univariate indices, and community structure were determined for each sampling unit using PERMANOVA+ (see the Methods section in the main report). Similarly, the probablities of

significant differences between sulfide classes were calculated for the univariate indices and community structure.

Relationships between sulfide concentration (ln-transformed) and various biodiversity measures were determined for different sampling units using the DISTLM routine of PERMANOVA+ (in PRIMER v6). For community structure, PERMANOVA+ was run using the resemblance matrix of Bray-Curtis similarities for square root transformed abundance data. For each relationship, the probability (p) and proportion of variation explained (Prop.) were determined. Relationships could not be determined using the "site" sampling units, due to the lack of replicates per site.

Results

Effects of sampling unit size on sulfide variability

The sulfide variability (as indicated by mean SDs and CVs at each site) increased as the sampling unit size increased (Fig. C1). The increases were smallest at reference site C.

Effects of sampling unit size on diversity indices

The number of individuals (as well as the number of *Capitella* spp. and *Ennucula/Nucula*) and other univariate diversity indices calculated using sampling units with different sizes (derived by combining data from individual grab samples at each site) are shown in Fig. C2-C4. The sampling unit size had a large effect on the mean values per site for the number of taxa (*T*) and Margalef's species richness index (*d*); some effect on the Shannon diversity index (*H'*), especially at site B; and relatively little effect on the number of individuals (*N*), the number of *Capitella* spp., the number of *Ennucula/Nucula*, and Pielou's evenness index (*J'*). The decreases observed in the mean numbers of individuals (*N*) and *Capitella* spp., when going from the 'grab' to 'station' sampling units at site A were mainly due to high numbers of *Capitella* spp. (720 m^{-2}) in one grab at station A6. Comparing sites, the changes in diversity indices with increasing sampling unit size were least at reference site C (except for *J'*). The curves for the number of taxa (*T*) and Margalef's species richness index (*d*) did not level off at the larger sampling units.

Using individual grabs as the sampling units, there were significant differences among sites ($p<0.05$) for all of the univariate diversity indices, except the abundance of *Ennucula/Nucula* (Table C1). Using stations as the sampling units, there were also significant differences ($p<0.05$) among sites for all of the indices, except the abundance of *Ennucula/Nucula*. Using station pairs as the sampling unit, there were significant differences among sites ($p<0.05$) for the number of individuals (*N*), the number of taxa (*T*), and the number of *Capitella* spp., but not for the other indices.

Using individual grabs as the sampling units, there were significant differences among sulfide classes ($p<0.05$) for all of the univariate diversity indices, except the number of individuals (*N*), the abundance of *Capitella* spp., and the abundance of *Ennucula/Nucula* (Table C2). Using stations as the sampling units, there were significant differences ($p<0.05$) among sites for all of the indices, except the abundance of *Ennucula/Nucula*. Using station pairs as the sampling unit, there were significant differences among sites ($p<0.05$) only for Pielou's index (*J'*).

The stress levels in the 2-dimensional MDS plots were all ≤0.1, indicating good ordination. There were clear differences in the MDS plots among the different sampling units (Fig. C5). For the community structure data, there were significant differences among sites and among sulfide classes ($p<0.05$) for grabs, stations, and station pairs (Tables C1 & C2).

Effects of sampling unit size on the relationships between sulfide concentration and diversity measures

Using individual grabs as the sampling units, there were significant relationships between sulfide concentration (ln-transformed) and each of the biodiversity measures, except for the number of individuals and the abundance of *Ennucula/Nucula* (Table C3). Using stations as the sampling units, there were significant relationships for *H'*, *J'*, abundance of *Capitella* spp., and community structure. Using station pairs as the sampling units, the only significant relationships were for *J'* and community structure.

Discussion

A 'species accumulation curve' can be used to describe the cumulative number of species (or taxa) observed as a function of a measure of effort (such as the surface area of the samples) in an area of habitat that is roughly homogeneous (Colwell & Coddington 1994). Such a curve can be used to determine the minimum sampling area that will ensure adequate taxonomic coverage for a habitat. However, the sampling effort required to reach the asymptote of accumulation curves is rarely reached (especially in marine sediments), so complete taxonomic coverage is usually not obtained (Clarke & Warwick 2001; Ugland et al. 2003).

The graph of the number of taxa vs. sampling unit size in our study (Fig. C2 bottom) is equivalent to a 'species accumulation curve'; however, it does not strictly meet the criterion of habitat homogeneity, because, as indicated by the sulfide variability data (Fig. C1), the habitat heterogeneity increased as the sampling unit size increased. This was likely because, as the sampling unit size increased, so did the geographic coverage of the sampling units, due to the consolidation of data from different stations in the larger sampling units. Thus the changes we observed in some univariate diversity indices as the sampling unit size increased were likely not entirely the result of increased sampling effort within a homogeneous habitat, but also due to increased heterogeneity in the habitat sampled in the larger sampling units. Therefore, combining data from more than one grab (especially from more than one station) may not be justified as a way of ensuring better taxonomic coverage within a habitat type, especially at the two farm sites, where there was greater habitat (sulfide) heterogeneity among stations, compared to the more homogenous conditions at the reference site.

Another problem with combining grab samples into larger sampling units is the loss of analytical capability. When going from 'grabs' to 'stations', we increase the sampling unit size three-fold, but we no longer have replicate samples at each station. With larger sampling unit sizes, we have fewer data points with which to make comparisons among sites or sulfide classes, or to examine the relationship with sulfide concentration. Also, for the data points that remain, each represents greater habitat (sulfide) heterogeneity. As a result, we are less able to show differences among

sites and sulfide classes: there were fewer diversity measures showing significant differences among sites for the station pair sampling units, compared to stations and grabs. Similarly, we are less able to define the relationships between biodiversity and sulfide concentration: there were fewer diversity measures showing significant relationships for the station pair sampling units compared to stations, and fewer for stations compared to grabs.

In our study of marine sediments affected by fish farming, *H'* increased non-linearly with increasing sampling unit size (especially at smaller sizes), but there was relatively little change in *J'* with sampling unit size. Similar results were found in a study on the effect of sampling quadrat size on *H'* and *J'* in a terrestrial plant community (Kwiatkowska & Symonides 1986). However, in a study of marine sediments affected by an oil spill, Clarke & Warwick (2001) found that the values of most diversity indices were sensitive to sample size.

If we want to increase sampling effort within roughly homogeneous habitats in our study (to better ensure complete taxonomic coverage within each habitat), we would need to collect larger samples at each station, either by using samplers that can collect larger individual samples (e.g. larger grabs or cores) or by taking more replicate samples (which could be consolidated into larger samples) at each station. In this way, we could increase the sampling unit size, without substantially increasing the habitat heterogeneity of the sampling unit.

It appears that using individual grab samples would clearly underestimate the number of taxa and consequently affect estimates of other biodiversity indices. As the sample unit size increased, the number of taxa increased, but some of this increase may be due to increased habitat heterogeneity in the sample units. Also, as sampling unit size increased, the number of samples decreased, thus reducing the ability to examine the relationships between sulfide concentration and biodiversity. As a compromise, we chose the consolidated abundance data from the triplicate grab samples at each station as our unit for calculating biodiversity indices. By combining the triplicate samples, we no longer have replicate sampling at each station, thus reducing some statistical power; by not consolidating sampling units above the "station" unit, we limit the heterogeneity within each sampling unit and we maintain 6 data points per site for examining the relationship between sulfide concentration and biodiversity.

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Table C1. Probabilities for significance tests comparing 3 study sites (2 fish farms and a reference site) for sulfide concentration and various diversity measures, using different sampling units. For sulfide concentration, univariate diversity indices, and selected taxa, permutational analyses of variance were computed using PERMANOVA+ for PRIMER (using resemblance matrices of Euclidean distances among samples for each measure). For community structure, PERMANOVA+ was run on the resemblance matrix of Bray-Curtis similarities for square root transformed abundance data. Probabilities in bold italics are significant ($p<0.05$).

	Sampling unit		
Diversity measure	Grabs	Stations	Station pairs
Sulfide (ln-transformed)	***<0.01***	***<0.01***	***<0.01***
Univariate diversity indices			
No. of individuals (*N*)	***<0.01***	***<0.01***	***0.02***
No. of taxa (*T*)	***<0.01***	***<0.01***	***<0.05***
Shannon (*H'*)	***<0.01***	***<0.01***	0.11
Margalef (*d*)	***<0.01***	***<0.01***	0.09
Pielou (*J'*)	***<0.01***	***<0.01***	0.17
Selected taxa			
No. of *Capitella* spp.	***<0.01***	***0.02***	***<0.05***
No. of *Ennucula/Nucula*	0.46	0.93	0.81
Community structure	***<0.01***	***<0.01***	***<0.01***

Table C2. Probabilities for significance tests comparing sulfide classes for various diversity measures, using different sampling units, in a study at 3 sites (2 fish farms and a reference site). For univariate diversity indices and selected taxa, permutational analyses of variance were computed using PERMANOVA+ for PRIMER (using resemblance matrices of Euclidean distances among samples for each measure). For community structure, PERMANOVA+ was run on the resemblance matrix of Bray-Curtis similarities for square root transformed abundance data. Probabilities in bold italics are significant ($p<0.05$).

	Sampling unit		
Diversity measure	Grabs	Stations	Station pairs
Univariate diversity indices			
No. of individuals (*N*)	0.06	***0.02***	0.10
No. of taxa (*T*)	***<0.01***	***<0.01***	0.14
Shannon (*H'*)	***<0.01***	***<0.01***	0.10
Margalef (*d*)	***<0.01***	***<0.01***	0.14
Pielou (*J'*)	***<0.01***	***0.01***	***0.03***
Selected taxa			
No. of *Capitella* spp.	0.08	***<0.01***	0.11
No. of *Ennucula/Nucula*	0.08	0.37	0.60
Community structure	***<0.01***	***<0.01***	***0.01***

Table C3. Probabilities (p) and proportions of variation explained (Prop.) for the relationships between sulfide concentration (ln-transformed) and various biodiversity measures using different sampling units, in a study at 3 sites (2 fish farms and a reference site). Relationships were determined using the DISTLM routine of PERMANOVA+ (in PRIMER v6). For community structure, PERMANOVA+ was run using the resemblance matrix of Bray-Curtis similarities for square root transformed abundance data. Probabilities in bold italics are significant ($p<0.05$).

	Sampling unit					
	Grabs		Stations		Station pairs	
Diversity measure	p	Prop.	p	Prop.	p	Prop.
No. of individuals (*N*)	0.88	<0.01	0.65	0.01	0.97	<0.01
No. of taxa (*T*)	***<0.01***	0.23	0.11	0.15	0.72	0.02
Shannon (*H'*)	***<0.01***	0.44	***<0.01***	0.42	0.15	0.28
Margalef (*d*)	***<0.01***	0.30	0.05	0.22	0.61	<0.01
Pielou (*J'*)	***<0.01***	0.30	***<0.01***	0.46	***0.02***	0.45
No. of *Capitella* spp.	***0.02***	0.08	***0.02***	0.30	0.07	0.37
No. of *Ennucula/Nucula*	0.48	0.01	0.79	0.01	0.74	0.02
Community structure	***<0.01***	0.29	***<0.01***	0.35	***<0.01***	0.35

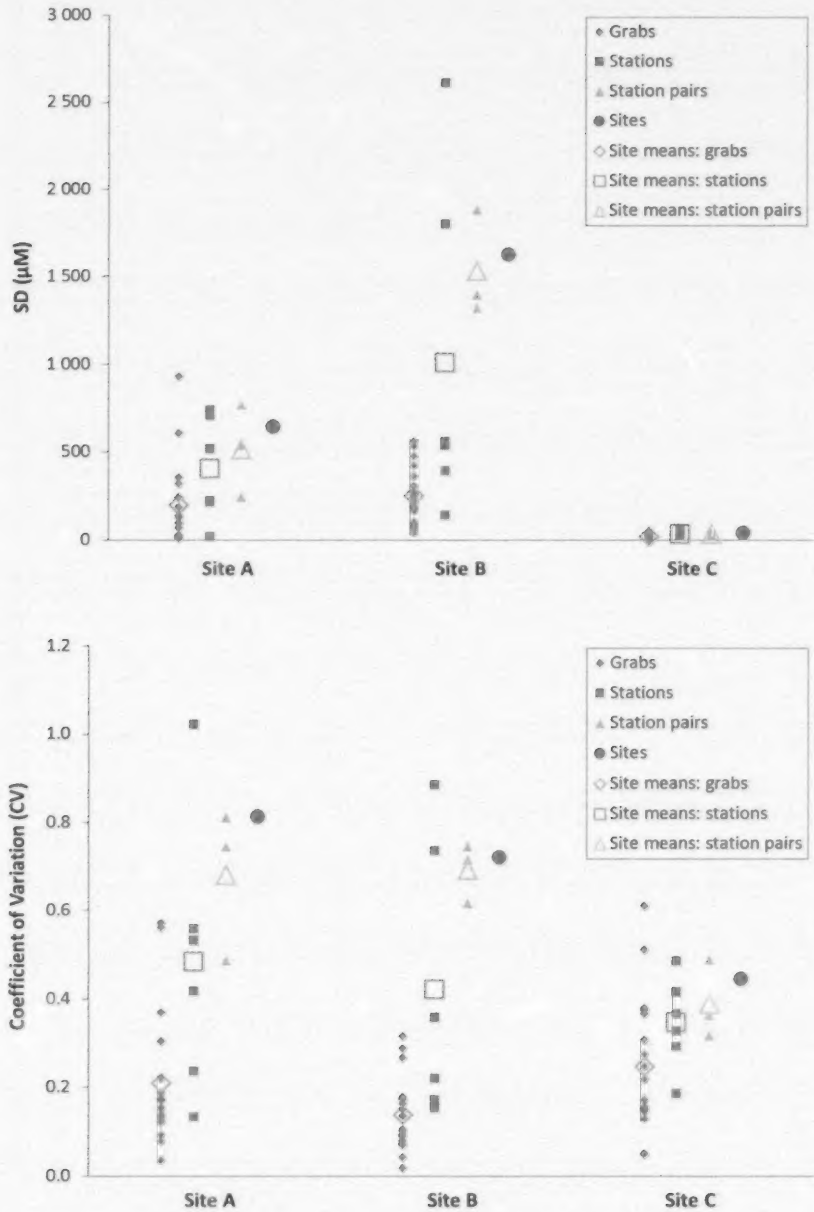

Fig. C1. Variability in sulfide measurements in different sampling units derived through consolidation of grab samples at 2 fish farms (sites A & B) and a reference site (C). Top: standard deviation (SD). Bottom: coefficient of variation (CV=SD/mean). Values for each sampling unit are shown, as well as site means (for each size of sampling unit). The sampling units are: grabs (18 grabs per site; 3 sulfide measurements per grab); stations (6 stations per site; 9 measurements per station); station pairs (3 station pairs per site; 18 measurements per station pair); and sites (1 sampling unit per site; 54 measurements per site).

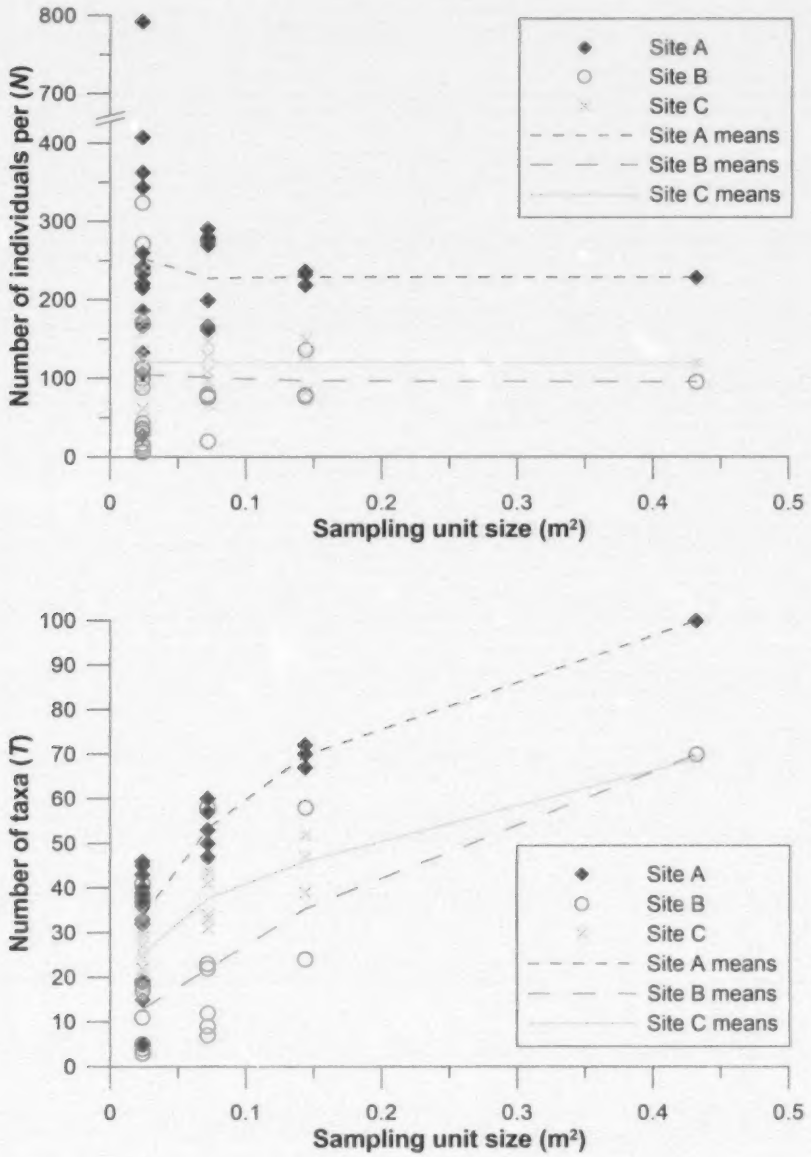

Fig. C2. Effect of sampling unit size on the number of individuals per kg (wet weight) of sediment (*N*) (top) and the number of taxa (*T*) (bottom) for benthic macrofauna collected at two salmon farms (sites A & B) and a reference site (C). The x-axis represents the surface area of the sampling units: 0.024 m^2 per grab sample (n=18 per site); 0.072 m^2 per station (3 grab samples combined per station; n=6 per site); 0.144 m^2 per station pair (6 grab samples combined per station pair; n=3 per site); and 0.432 m^2 per site (18 grab samples combined per site; n=1 per site). Lines connect mean values for each sampling unit size at each site.

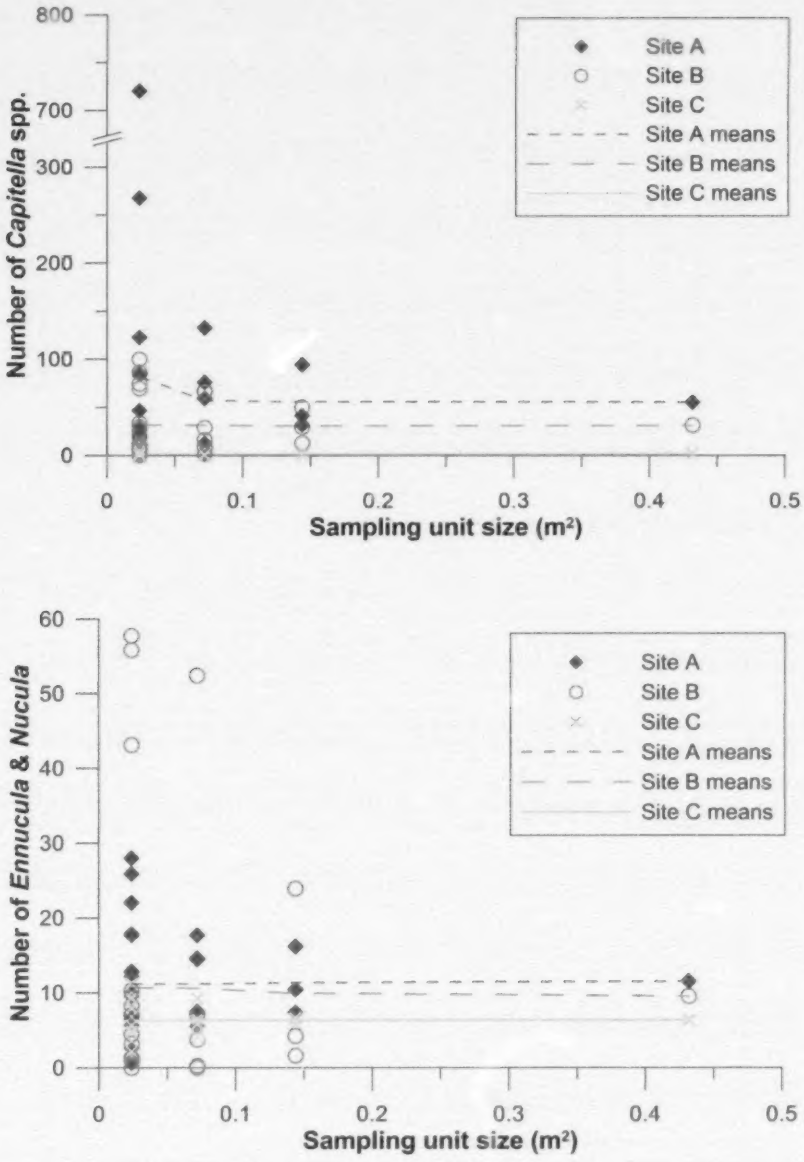

Fig. C3. Effect of sampling unit size on the number of *Capitella* spp. (top) and the number of *Ennucula delphinodonta* and *Nucula proxima* combined (bottom) collected at two salmon farms (sites A & B) and a reference site (C). Abundance data are per kg (wet weight) of sediment. The x-axis represents the surface area of the sampling units: 0.024 m^2 per grab sample (n=18 per site); 0.072 m^2 per station (3 grab samples combined per station; n=6 per site); 0.144 m^2 per station pair (6 grab samples combined per station pair; n=3 per site); and 0.432 m^2 per site (18 grab samples combined per site; n=1 per site). Lines connect mean values for each sampling unit size at each site.

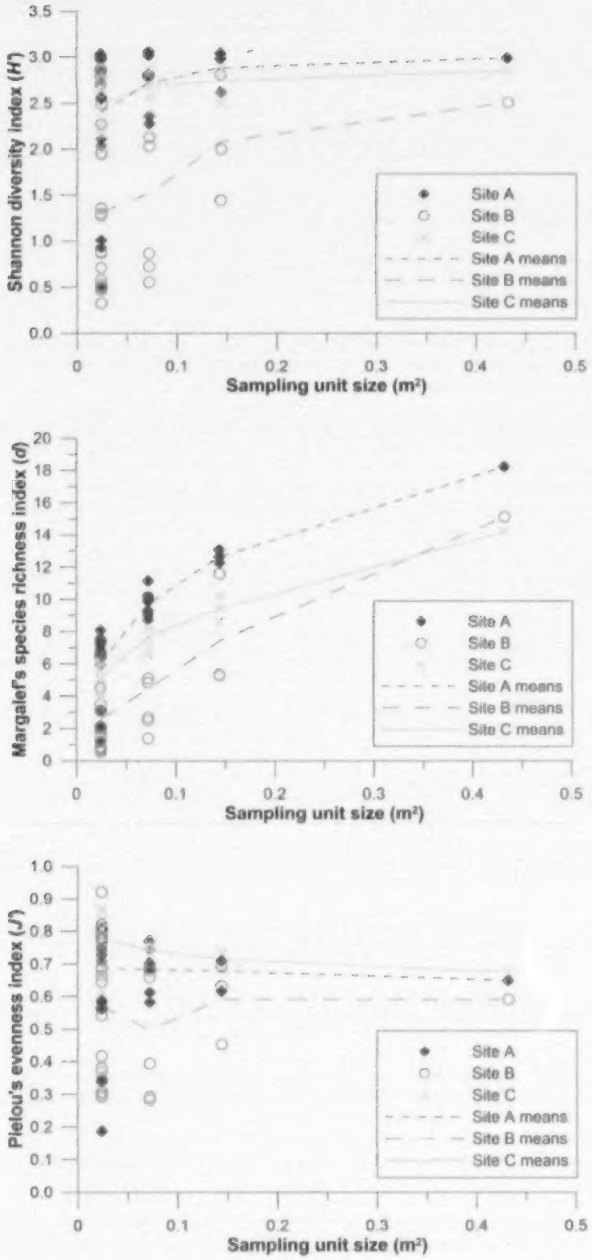

Fig. C4. Effect of sampling unit size on the Shannon diversity index (H') (top), Margalef's species richness index (d) (middle), and Pielou's evenness index (J') (bottom) for benthic macrofauna collected at two salmon farms (sites A & B) and a reference site (C). The x-axis represents the surface area of the sampling units: 0.024 m^2 per grab sample (n=18 per site); 0.072 m^2 per station (3 grab samples combined per station; n=6 per site); 0.144 m^2 per station pair (6 grab samples combined per station pair; n=3 per site); and 0.432 m^2 per site (18 grab samples combined per site; n=1 per site). Lines connect mean values for each sampling unit size at each site.

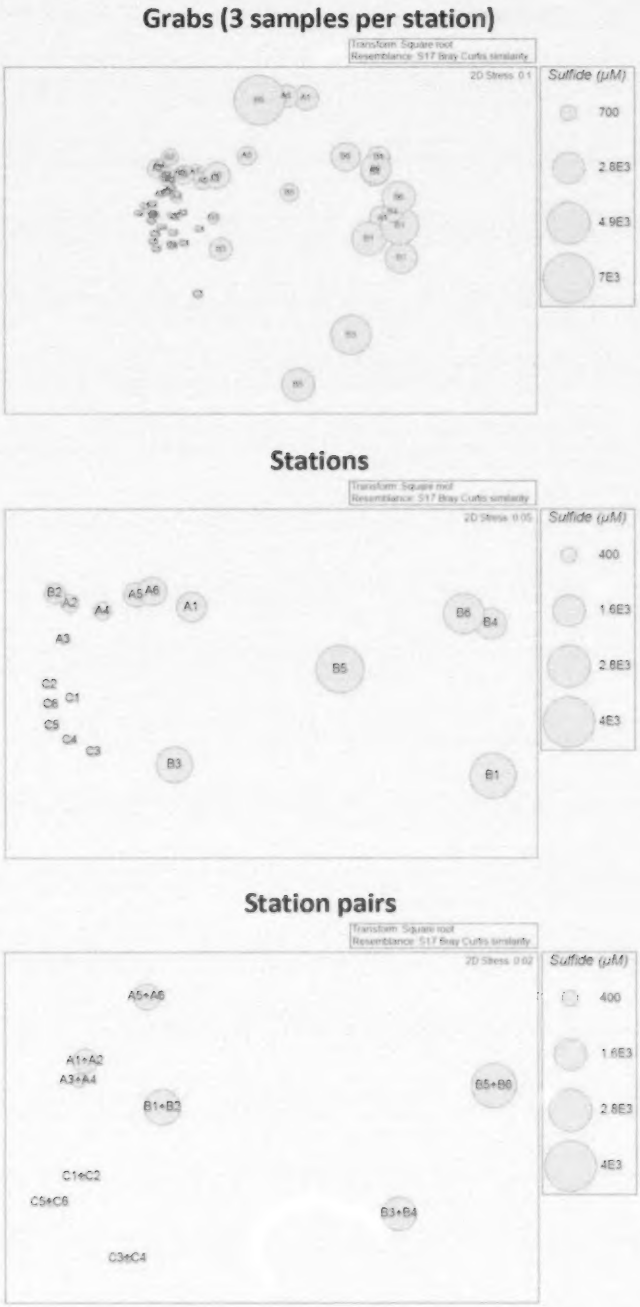

Fig. C5. Effect of sampling unit size on multi-dimensional scaling (MDS) bubble plots of benthic macrofaunal abundance data (square-root transformed) in sediment samples collected at two farms sites (A & B) and a reference site (C). MDS plots were produced using PRIMER v6 software. Plots were calculated using abundance data for each triplicate grab sample (top); total abundance per station (middle); and total abundance per station pair (bottom). Circle sizes are in proportion to mean sulfide concentrations (see legend at right).

EFFECT OF USING HIGHER TAXONOMIC LEVELS ON DIVERSITY MEASURES

Introduction

Clarke & Warwick (2001) noted that, in many marine pollution studies, it has been found that there is little or no loss of information when species are aggregated at higher taxonomic levels. In our study, 115 of the 123 taxa were identified to the species level. The lowest taxonomic levels (i.e. species in most cases) were used to calculate univariate and multivariate diversity measures in the main study. In this appendix, we examined the effect of using aggregations to higher taxonomic levels on various diversity measures.

Methods

Calculations of univariate diversity indices and MDS plots were repeated with the originally identified taxa aggregated to higher taxonomic levels: genus, family, order, class, and phylum. Taxonomic classifications are from the World Register of Marine Species (WoRMS Editorial Board 2016); except where no classification was provided for a given level, the classification in the Integrated Taxonomic Information System on-line database (ITIS 2016) was used (see Appendix B). Refer to the main report for methods used to calculate the various diversity measures.

Results

Table D1 shows the number of taxa at different levels of aggregation. Note that each aggregation level (except phylum) included a few taxa that were not identified to the aggregation level of interest (i.e. they were only identified to a higher taxonomic level). Although there were only 8 taxa (of a total 123) not identified to the species level, they represented 41% of the total number of individuals (all stations at all 3 sites combined); 20% was due to *Capitella* spp. (probably *C. capitata* and possibly 1 or 2 other species) and 14% was due to unidentified Tubificidae. The 7 taxa not identified to genus represented 20% of the total number (14% due to unidentified Tubificidae); the 3 taxa not identified to family represented 5% of the total number; and the 2 taxa not identified to order represented 4% of the total number.

The number of individuals per kg (wet weight) of sediment (N) remained the same (as expected), regardless of the taxonomic level; the values per station were the same as in Table 4 of the main report. Values for other univariate indices (number of taxa, T; Shannon, H'; Margalef, d; and Pielou, J') decreased overall as the taxonomic level increased; the decreases were generally small from species to family, but greater at higher taxonomic levels (Figs. D1 & D2).

The stress levels in the 2-dimensional MDS plots using taxa aggregated to different taxonomic levels were all ≤ 0.1, indicating good ordination (Fig. D3). As with the univariate indices, there was little change in the MDS plots from species to family, but greater differences at higher taxonomic levels. At higher taxonomic levels, the separation between sites became less clear, as did the relationship with sulfide concentration (Fig. D3).

Comparisons among sites were significant for *N*, *T*, and community structure at all aggregation levels (Table D2). Comparisons among sites for *H'*, *d*, and *J'* were significant at the species to order aggregation levels, but not for class and phylum (except *d* was significant at the phylum level) (Table D2).

Comparisons among sulfide classes were significant for *N* and community structure at all aggregation levels (Table D3). Comparisons among sulfide classes for *T* were significant at all aggregation levels, except phylum (Table D3). Comparisons among sulfide classes for *H'*, *d*, and *J'* were significant at the species to order aggregation levels, but not for class and phylum (Table D3).

Probabilities and proportions of variation explained for the relationships between sulfide concentration (ln-transformed) and various biodiversity measures, using different taxonomic levels, are shown in Table D4. Increases in probability levels (and decreases in proportions of variance explained) for the relationships occurred mainly at the class and phylum levels. The relationships for *N* and *T* were not significant at all aggregation levels. The relationships for *H'*, *J'*, and community structure were significant at all aggregation levels, except phylum. The relationship for *d* was significant only at the order level.

Discussion

In our study, analysis of community structure (based on resemblance matrices) showed significant differences among sites and among sulfide classes at all taxonomic levels. However, many of the univariate diversity indices showed significant differences among sites and among sulfide classes only at the species to order levels. Similarly, increases in significance (and decreases in the proportion of variance explained) for the relationships between sulfide concentration and biodiversity measures occurred mainly at the class and phylum levels. This suggests that identification to the family (and possibly to the order) should be sufficient for demonstrating differences among fish farm sites in SWNB, as has been found in other marine microbenthic pollution studies (e.g. Warwick 1988; Clarke & Warwick 2001). Identification only to the family level should result in savings in costs and time.

References

Clarke, K.R., and Warwick, R.M. 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd ed. PRIMER-E, Plymouth, U.K.

ITIS (Integrated Taxonomic Information System). 2016. ITIS on-line database. Available from: https://www.itis.gov/ (accessed September 2016).

Warwick, R.M. 1988. The level of taxonomic discrimination required to detect pollution effects on marine benthic communities. Mar. Poll. Bull. 19: 259-268.

WoRMS Editorial Board. 2016. World Register of Marine Species. Vlaams Instituut voor de Zee (Flanders Marine Institute), Oostende, Belgium.
Available from: http://www.marinespecies.org/ (accessed September 2016).

Table D1. Number of taxa based on original identification data (mostly to the species level) and aggregation to genus, family, order, class, and phylum. Benthic macrofaunal abundance data were obtained from grab samples collected at 3 sites (2 fish farms and a reference site). See Appendix B for a list of taxa and their classifications.

Taxonomic aggregation level	Number of pre-aggregation taxa	Number of pre-aggregation taxa identified to the indicated taxonomic level	Number of different taxa at the indicated taxonomic aggregation level	Number of pre-aggregation taxa not identified to the indicated aggregation level
Species	123	115	115	8
Genus	123	116	107	7
Family	123	120	86	3
Order	123	121	44	2
Class	123	123	23	0
Phylum	123	123	11	0

Table D2. Probabilities for significance tests comparing 3 study sites (2 fish farms and a reference site) for various diversity measures, calculated using taxa aggregated to different taxonomic levels. For univariate diversity indices, permutational analyses of variance (2-way nested: stations within sites) were computed with PERMANOVA+ for PRIMER (using resemblance matrices of Euclidean distances among samples for each measure). For community structure, PERMANOVA+ was run on the resemblance matrix of Bray-Curtis similarities for square root transformed abundance data. Probabilities in bold italics are significant ($p<0.05$).

	Taxonomic level					
Diversity measure	Species	Genus	Family	Order	Class	Phylum
No. of individuals (*N*)	***<0.01***	***0.01***	***0.01***	***0.01***	***0.01***	***0.01***
No. of taxa (*T*)	***<0.01***	***<0.01***	***<0.01***	***<0.01***	***<0.01***	***<0.01***
Shannon (*H'*)	***<0.01***	***<0.01***	***<0.01***	***0.01***	0.14	0.58
Margalef (*d*)	***<0.01***	***<0.01***	***<0.01***	***<0.01***	0.07	***0.03***
Pielou (*J'*)	***<0.01***	***<0.01***	***0.01***	***0.03***	0.08	0.58
Community structure	***<0.01***	***<0.01***	***<0.01***	***<0.01***	***<0.01***	***<0.01***

Table D3. Probabilities for significance tests comparing sulfide classes for various diversity measures, calculated using taxa aggregated to different taxonomic levels, in a study at 3 sites (2 fish farms and a reference site). For univariate diversity indices, permutational analyses of variance (2-way nested: stations within sites) were computed with PERMANOVA+ for PRIMER (using resemblance matrices of Euclidean distances among samples for each measure). For community structure, PERMANOVA+ was run on the resemblance matrix of Bray-Curtis similarities for square root transformed abundance data. Probabilities in bold italics are significant ($p<0.05$).

	Taxonomic level					
Diversity measure	Species	Genus	Family	Order	Class	Phylum
No. of individuals (*N*)	***0.02***	***0.03***	***0.03***	***0.03***	***0.03***	***0.03***
No. of taxa (*T*)	***<0.01***	***<0.01***	***<0.01***	***<0.01***	***<0.05***	0.14
Shannon (*H'*)	***<0.01***	***<0.01***	***<0.01***	***<0.01***	0.06	0.65
Margalef (*d*)	***<0.01***	***<0.01***	***<0.01***	***<0.01***	0.29	0.36
Pielou (*J'*)	***0.01***	***0.01***	***0.02***	***0.03***	0.05	0.49
Community structure	***<0.01***	***<0.01***	***<0.01***	***<0.01***	***<0.01***	***<0.01***

Table D4. Probabilities (p) and proportions of variation explained (Prop.) for the relationships between sulfide concentration (ln-transformed) and various biodiversity measures calculated using taxa aggregated to different taxonomic levels, in a study at 3 sites (2 fish farms and a reference site). Relationships were determined using the DISTLM routine of PERMANOVA+ (in PRIMER v6). For community structure, PERMANOVA+ was run using the resemblance matrix of Bray-Curtis similarities for square root transformed abundance data. Probabilities in bold italics are significant ($p<0.05$).

	Taxonomic level					
	Species		Genus		Family	
Diversity measure	p	Prop.	p	Prop.	p	Prop.
No. of individuals (*N*)	0.65	0.01	0.65	0.01	0.65	0.01
No. of taxa (*T*)	0.11	0.15	0.12	0.14	0.11	0.15
Shannon (*H'*)	***<0.01***	0.42	***<0.01***	0.41	***<0.01***	0.41
Margalef (*d*)	0.05	0.22	0.07	0.20	0.06	0.22
Pielou (*J'*)	***<0.01***	0.46	***<0.01***	0.45	***<0.01***	0.43
Community structure	***<0.01***	0.35	***<0.01***	0.35	***<0.01***	0.35

	Taxonomic level					
	Order		Class		Phylum	
Diversity measure	p	Prop.	p	Prop.	p	Prop.
No. of individuals (*N*)	0.65	0.01	0.64	0.01	0.64	0.01
No. of taxa (*T*)	0.08	0.19	0.47	0.03	0.74	0.01
Shannon (*H'*)	***<0.01***	0.38	***0.03***	0.25	0.55	0.02
Margalef (*d*)	***0.03***	0.27	0.70	0.01	0.25	0.08
Pielou (*J'*)	***<0.01***	0.36	***0.02***	0.30	0.29	0.07
Community structure	***<0.01***	0.25	***0.02***	0.17	0.19	0.09

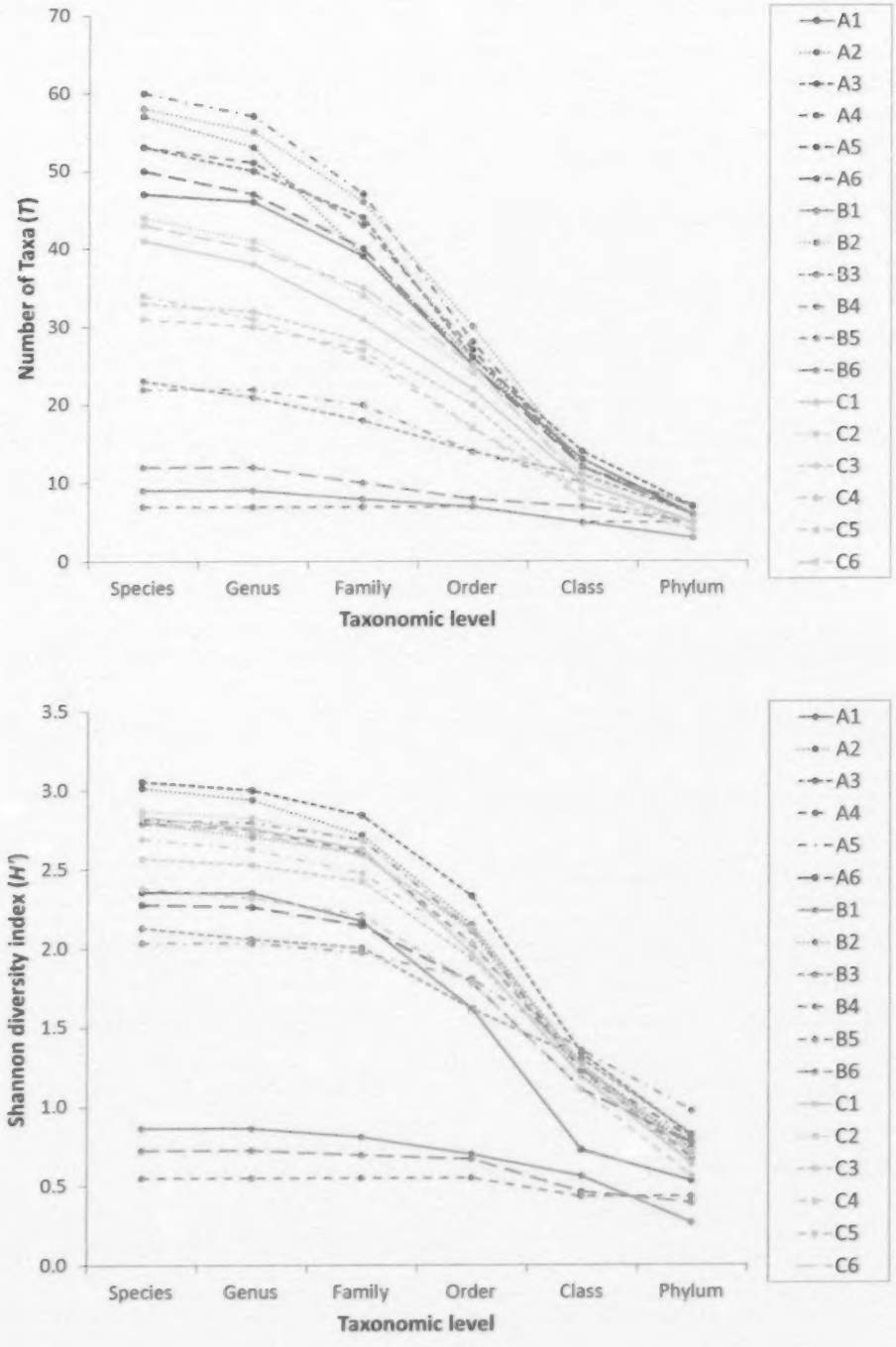

Fig. D1. Values for the number of taxa (*T*) (top) and the Shannon diversity index (*H'*) (bottom) calculated using taxa aggregated to species, genus, family, order, class, and phylum. Benthic grab samples were collected at 3 sites (fish farm sites A & B and reference site C), 6 stations per site.

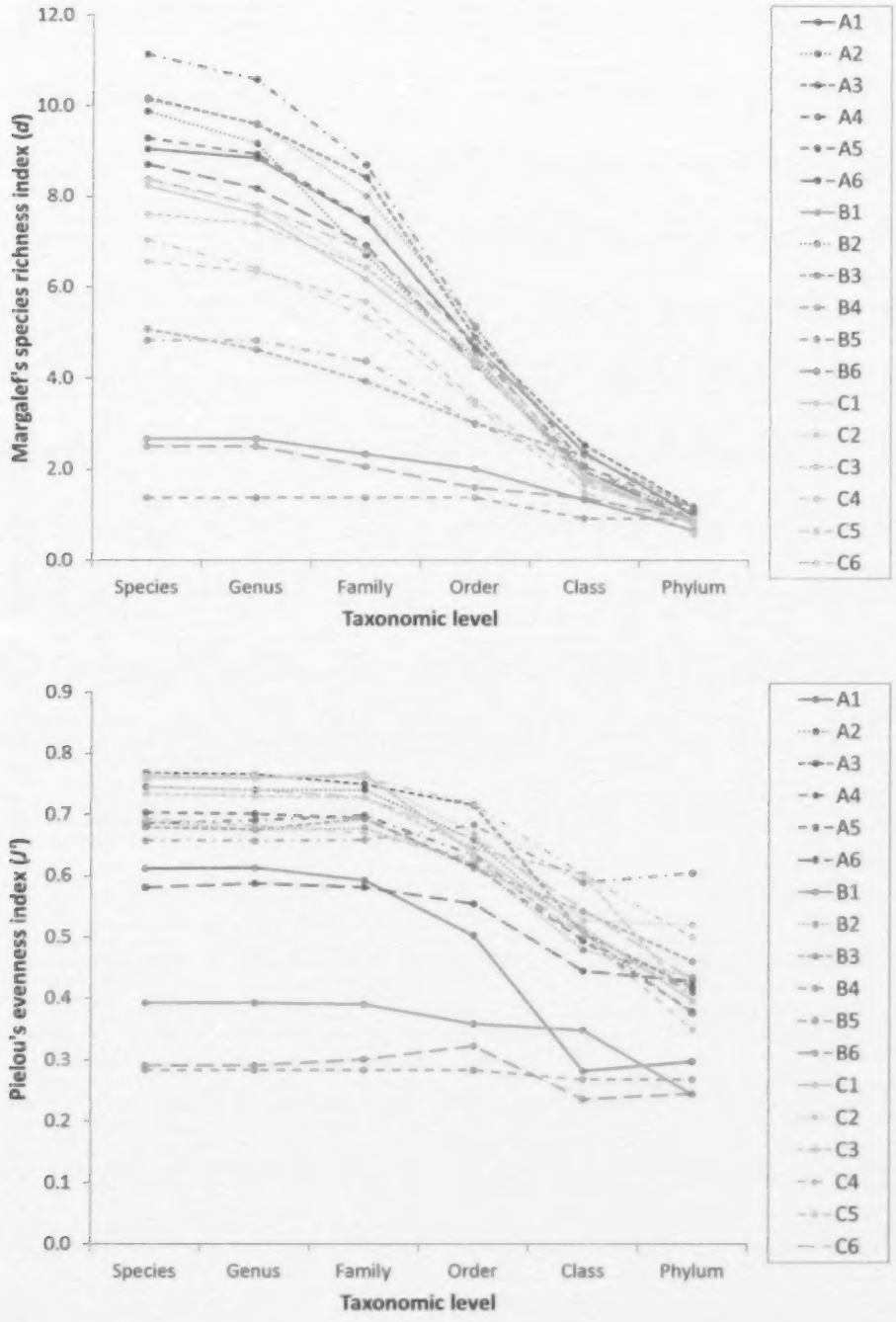

Fig. D2. Values for Margalef's species richness index (*d*) (top) and Pielou's evenness index (*J'*) (bottom) calculated using taxa aggregated to species, genus, family, order, class, and phylum. Benthic grab samples were collected at 3 sites (fish farm sites A & B and reference site C), 6 stations per site.

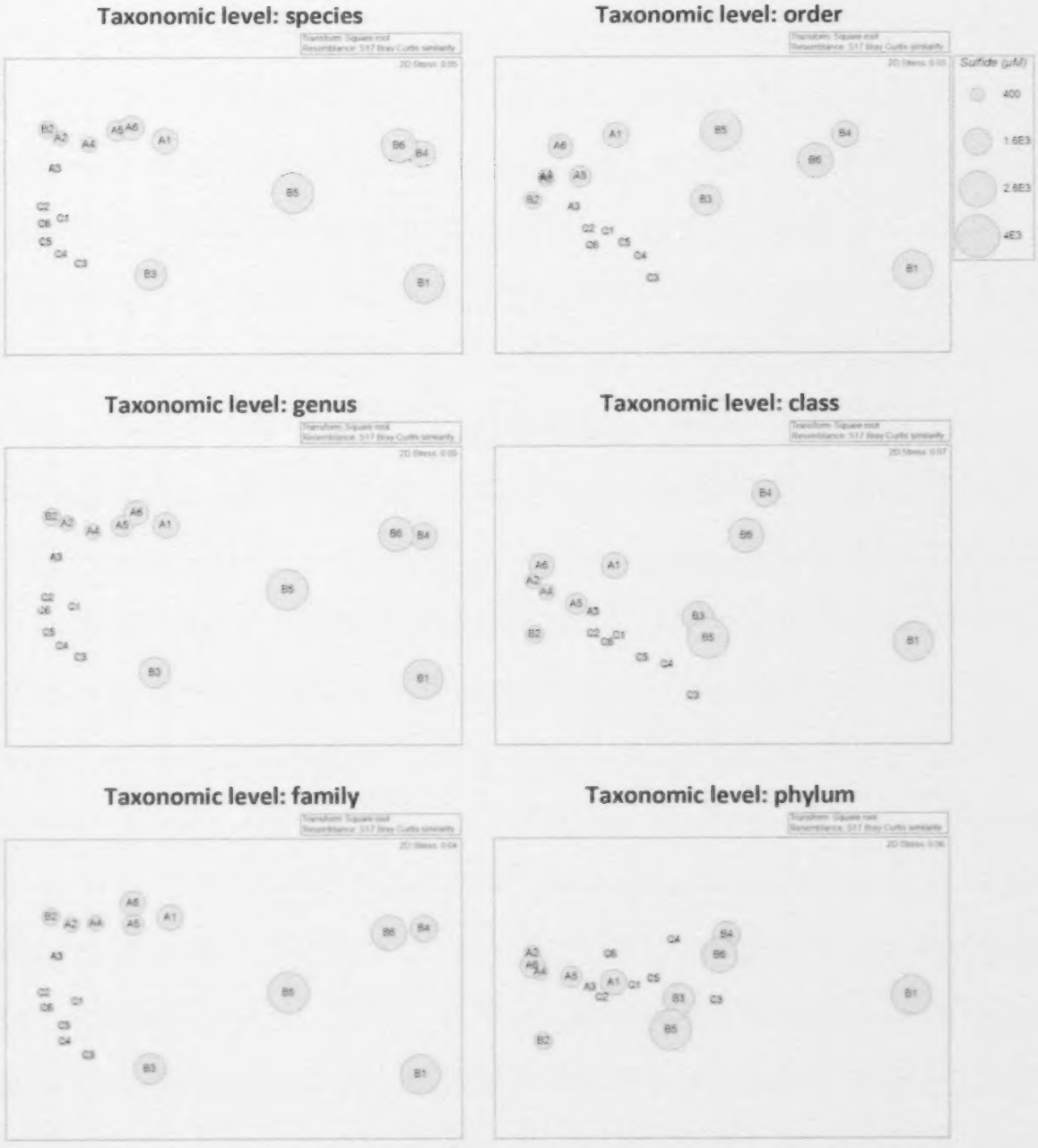

Fig. D3. Effect of taxonomic level on multi-dimensional scaling (MDS) bubble plots of benthic macrofaunal abundance data (square-root transformed) in sediment samples collected at two farms sites (A & B) and a reference site (C). MDS plots were produced using PRIMER v6 software. Plots were calculated using abundance data aggregated to species, genus, family, order, class, and phylum. Circle sizes are in proportion to mean sulfide concentrations (see legend at top right). Abundance data were combined from triplicate grab samples at each of 6 stations per site.